Assurance® GDS *E. coli* O157:H7 Tq

There are two validated methods that can be followed:

AOAC® *Official Method of Analysis*℠ 2005.04

Health Canada Method MFLP-16

Part No: 71007-100 (100 tests)
71007-576 (576 tests)
71007-576ATM (576 tests)

General Description

Assurance® GDS for *E. coli* O157:H7 Tq is an automated nucleic acid amplification system for the detection of pathogenic *E. coli* O157:H7/NM in foods, ingredients, carcass cloths, and environmental samples. Assurance® GDS for *E. coli* O157:H7 Tq utilizes a proprietary IMS-based sample preparation procedure to capture organisms belonging to O157 serogroup prior to genetic analysis for the associated pathogenicity genes. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

Kit Components

Each Assurance® GDS for *E. coli* O157:H7 Tq kit (**100** and **576** tests) contains the following:

- Amplification Tubes Tq
- O157 Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

Each Assurance® GDS for *E. coli* O157:H7 Tq **576ATM** kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary for **576ATM** kit but sold separately:

61031-100	Wash Solution Kit
34724-100C	Resuspension Buffer Tq

Equipment / Materials Required

Other necessary materials not provided include:

- mEHEC® media
- Assurance® GDS Rotor-Gene® thermocycler
- GDS rotor and locking ring
- Laptop computer and software v.2.3.103
- PickPen® and PickPen® tips
- Vortex mixer (IKA® MS 3, or equivalent)
- Adhesive film strips
- GDS sample wells and sample wells base
- Resuspension plate
- Stomacher® paddle homogenizer, or equivalent
- Stomacher®-type bags with filter, or equivalent
- 8-channel micropipette capable of accurately dispensing 30 µL

Repeat pipette
Adjustable micropipette
Repeat pipette tips (0.5 mL and 10 mL)
Filter barrier micropipette tips (50 µL and 1.0 mL)
Gel cooling block
Incubator capable of maintaining 42 ± 1 °C
Freezer capable of maintaining -20 ± 5 °C
Refrigerator capable of maintaining 5 ± 3 °C

Additional materials for the **576** test kit include (AOAC method only):

Variable spacing amp tube holder, 72 well
Variable spacing amp tube holder lid, 72 well
Amp tube capping tool
Amp tube cap rack, 72 well
Aluminum cooling block, 72 well
72 well rotor and locking ring

For information on additional materials needed for sample analysis by the Assurance® GDS PickPen® PIPETMAX® (PPMX) please see the PPMX User Manual (No. 55240 / 20516473).

AOAC® OFFICIAL METHOD OF ANALYSIS℠ 2005.04

Approved matrices include: raw ground beef, raw beef trim, frozen finely textured beef, orange juice, apple juice, leaf lettuce, green onions, MicroTally™ carcass cloth, and sprout process water

Sample Preparation

Note: For this method when a temperature of 42 °C is specified the acceptable temperature range is 42 ± 1 °C.

A. Enrichment Media Preparation

a. For 25 g sample, pre-warm 225 mL sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 7.1 g of mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared broth within 6 h.

b. For 375 g sample, pre-warm 1500 mL sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 47.3 g of mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared broth within 6 h.

c. Alternatively, mEHEC® media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to dissolve the powder, dispense into desired volume and autoclave at 121 °C for 15 min. Broth must be pre-warmed to 42 °C overnight prior to sample addition.

B. Test Portion Preparation and Enrichment

1. **25 g sample** – Add 25 g of sample to 225 mL pre-warmed (42 °C) mEHEC broth. If necessary, masticate or homogenize sample by hand for 2 min. Incubate for 6.5 – 18 h at 42 °C. For sprout irrigation water, incubate for 8 – 18 h. For green onions, incubate for 8 – 16 h. For juice, incubate for a minimum of 18 – 24 h.

2. **375 g sample of beef trim, ground beef, finely textured beef, or leafy greens** – Add 375 g of sample to 1500 mL pre-warmed (42 °C) mEHEC broth. If necessary, masticate or homogenize sample by hand for 2 min. Incubate for 8 – 18 h at 42 °C. For frozen state finely textured beef, incubate for a minimum of 10-18 h.

3. **Carcass cloths** – Use Fremonta MicroTally™ (https://www.fremonta.com/microtally) cloth or equivalent for sampling. Collect carcass cloth per FSIS (FSIS Directive 10,010.1 Rev. 4). Add 200 mL pre-warmed (42 °C) mEHEC broth to cloth in sample bag. Masticate or mix sample by hand for 2 min. Incubate for 8 – 16 h at 42 °C.

Note: Contact Technical Services (BioMTS@milliporesigma.com) for recommended procedures for testing alternate sample sizes.

C. Sample Extraction Protocol

Change gloves prior to handling reagents.

Note: Sample prep can also be completed using the Assurance® GDS PickPen® PIPETMAX (PPMX); for automation setup procedures, please see the PPMX User Manual (No. 55240).

1. Vortex **O157 Concentration Reagent.** Immediately transfer 20 µL to each of the required number of GDS sample wells (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Cover sample wells with adhesive strips.
2. Transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) using a repeat pipette and a 10 mL tip. Cover sample wells with adhesive strips.
3. Add 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover prepared resuspension plate with adhesive film strips.
4. Carefully remove adhesive film from 1 strip of sample wells containing O157 Concentration Reagent. Following incubation, gently mix enriched presumptive positive samples by hand to ensure homogeneity. Add 1.0 mL of incubated sample to each sample well. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film prior to adding samples to a new strip. **Immediately return samples to 42 °C incubator for use during confirmation, if necessary**.
5. Place sealed sample wells containing O157 Concentration Reagent and sample on the vortex mixer and vortex at 900 rpm for 5 – 15 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.
6. Carefully remove and discard adhesive film from 1 strip of samples. Remove corresponding adhesive film from a strip of sample wells containing Wash Solution.
7. Load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert tips into the first strip of sample wells. Stir tips gently for 30 s while continually moving tips up and down from the surface to the bottom of the wells. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.
8. Transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Tap the PickPen® tips against the side of the sample wells to remove excess droplets of Wash Solution.
9. Remove the adhesive film from resuspension plate. Transfer PickPen® tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips.
10. Repeat steps (6) through (9) for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

HEALTH CANADA METHOD MFLP-16

Approved matrices include: raw ground beef, raw beef trim, orange juice, apple juice, leafy greens and sprout process water

Sample Preparation

Note: All media must be prewarmed to 40 ± 2 °C before sample enrichment.

Note: For this method when a temperature of 42 °C is specified the acceptable temperature range is 42 ± 1 °C.

A. Enrichment Media Preparation

1. For 25 g sample, pre-warm 225 mL sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 7.1 g of mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.
2. Alternatively, mEHEC® media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to dissolve the powder, dispense into desired volume and autoclave at 121 °C for 15 min. Media must be pre-warmed to 42 °C overnight prior to sample addition.

B. Test Portion Preparation and Enrichment

1. **25 g sample** – Weigh 25 g of sample to 225 mL pre-warmed (42 °C) mEHEC® broth. If necessary, masticate or homogenize sample by hand for 2 min. Incubate for 6.5 – 18 h at 42 °C. For sprout process water, incubate for a minimum of 8 – 18 h at 42 °C. For sample size other than 25 g, up to 64 g, maintain the ratio of 1 portion sample to 9 portions mEHEC® media.
2. **375 g sample** – Add 375 g of sample to 1500 mL pre-warmed (42 °C) mEHEC® broth. For sample size 65 g up to 374 g, maintain the ratio of 1 portion sample to 4 portion191s mEHEC® media. If necessary, masticate or homogenize sample by hand for 2 min. Incubate for 8 – 18 h at 42 °C.

C. Sample Extraction Protocol

Change gloves prior to handling reagents.

Note: Sample preparation can also be completed using the Assurance® GDS PickPen® PIPETMAX (PPMX); for automation setup procedures please see the PPMX User Manual (No. 55240).

1. Vortex **O157 Concentration Reagent.** Immediately transfer 20 µL to each of the required number of GDS sample wells (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Cover sample wells with adhesive film strips.
2. Transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) using a repeat pipette and a 10 mL tip. Cover sample wells with adhesive film strips.
3. Add 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover prepared resuspension plate with adhesive film strips.
4. Carefully remove adhesive film from 1 strip of sample wells O157 Concentration Reagent. Following incubation, gently mix enriched presumptive positive samples by hand to ensure homogeneity. Add 1.0 mL of incubated sample to each well containing O157 Concentration Reagent. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. **Immediately return samples to 42 °C incubator for use during confirmation, if necessary.**
5. Place sealed sample wells containing O157 Concentration Reagent and sample on the vortex mixer and vortex at 900 rpm for 5 – 15 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.
6. Carefully remove and discard adhesive film from 1 strip of samples. Remove corresponding adhesive film from a strip of sample wells containing Wash Solution.
7. Load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert the tips into the first strip of sample wells. Stir gently for 30 s while continually moving tips up and down from the surface to the bottom of the well. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.
8. Transfer PickPen® tips to corresponding sample wells containing Wash Solution. With tips submerged, gently swirl the PickPen® tips from side to side for 10 s (do not release particles into Wash Solution). Tap the PickPen® tips against the side of the sample wells to remove excess droplets of Wash Solution.
9. Remove the adhesive film from resuspension plate. Transfer PickPen® tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips.
10. Repeat steps (6) through (9) for all samples using new tips for each strip of samples.

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents.

Note: Amplification tube preparation can also be completed using the PPMX, for setup procedures please see the PPMX User Manual (No. 55240).

A. Preparation of Gel Cooling Block

1. Prior to initial use for **100** and **576ATM** test kits, the gel cooling block must be stored in the freezer (-20 ± 5 °C) for minimum 6 h. When frozen, the gel cooling block will change color from pink to purple. When not in use, the gel cooling block should continue to be stored upside-down at -20 ± 5 °C.

2. Between each use the gel cooling block should be returned to the freezer and stored upside-down until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

3. The 72-well aluminum cooling block is for use with the **576** test kit and should be stored in the refrigerator (5 ± 3 °C).

B. Preparation of Amplification Tubes

1. The Assurance® GDS Rotor-Gene® set up and data entry should be completed prior to transferring samples from the resuspension plate into the **Amplification Tubes Tq**.

2. Remove Amplification tubes Tq from foil pouch and place them in the frozen gel cooling block (aluminum cooling block for **576** test kit). Reseal pouch

3. For the **100** and **576ATM** kits, open Amplification Tubes Tq. Briefly pipette up and down resuspension solution to resuspend beads. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipette and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each Amplification Tube to ensure that the cap is securely sealed.

4. For the **576** test kit, place tubes into variable-spacing Amplification Tubes holder. Using Amplification Tubes capping tool, remove lids from Amplification Tubes. Place the lid device on top of the holder. Use the Amplification Tube holder blades to slice apart the Amplification Tubes. Separate fully the Amplification Tubes with the variable-spacing holder. Briefly pipette up and down resuspension solution to resuspend beads. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipettor and filter barrier tips. Remove the lid from the holder. Push together the Amplification Tubes with the holder. Cap Amplification Tubes using the Amplification Tubes capping tool. Visually inspect each tube to ensure that the cap is securely sealed.

5. Place Amplification Tubes Tq into Assurance® Rotor-Gene® in sequential order, beginning with position #1. For the **100** and the **576ATM** test kits, use the 36-well rotor and locking ring; for the **576** test kit, use the 72-well rotor and locking ring.

   Note: For **576** test kit, after loading Amplification Tubes in the rotor and securing with locking ring, contents should be thoroughly mixed by shaking with a snapping motion. See Application Note **2060 / MK_AN4551EN / MS_AN4551EN** for details.

6. Start Rotor-Gene® cycle. Refer to Assurance® GDS user manual (No. 55342 / 20516474) for detailed instructions on operating the Rotor-Gene® thermocycler.

   Note: The Assurance® GDS Rotor-Gene® must be started within 20 min after addition of the samples to the Amplification Tubes.

Results

Upon completion of the run, the Assurance® GDS Rotor-Gene® software will provide a results table. Each sample will be identified as **Positive**, **Negative**, or **No Amp**.

Positive: Samples are presumptive positive for *E. coli* O157:H7.

Negative: Samples are negative for *E. coli* O157:H7.

No Amp: Amplification did not occur. Repeat the test beginning from step **Sample Extraction Protocol**. If No Amp result repeats, contact Technical Services (BioMTS@milliporesigma.com).

No.	Color	Name	Result	Assay	Kit Lot Number
1		Sample 1	Positive	*E. coli* O157:H7 Tq	1234567
2		Sample 2	Negative	*E. coli* O157:H7 Tq	1234567
3		Sample 3	No Amp	*E. coli* O157:H7 Tq	1234567

Confirmation

AOAC Methods: Following 8 – 24 h enrichment in mEHEC at 42 °C, samples can be confirmed from the retained mEHEC® enrichment via the following:

1) Modified U.S. Department of Agriculture (USDA-FSIS) *Microbiology Laboratory Guidebook*, Chapter 5C.01
2) Modified U.S. Food and Drug Administrative (FDA) *Bacteriological Analytical Manual*, *Chapter* 4A
3) An aliquot of the mEHEC® enrichment may be confirmed for *E. coli* O157:H7/NM via Assurance® GDS for Shiga Toxin Genes (O157) Tq kit.
   An aliquot of the mEHEC® enrichment may be confirmed for *E. coli* O157:H7/NM via Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq kit (AOAC PTM 101901).

Note: Enriched samples can be stored at 2 – 8 °C (refrigeration) for up to 24 h prior to confirmation.

Health Canada Method: Following 8 – 20 h enrichment in mEHEC® at 42 °C, samples can be confirmed from the retained enrichment via the following:

1) Health Canada, *Compendium of Analytical Methods* (MFHPB-10)
2) Take a new 1 mL portion of the minimum 8 h incubated mEHEC® and follow MFLP-16, beginning at step 1. At step 3, 35 µL of wash buffer should be used instead of resuspension buffer. Proceed with steps 8.2.4 to 8.2.10 and plate using the confirmation media of MFHPB-10. If no typical colonies can be isolated from the 8h mEHEC enrichment, repeat the cultural confirmation, as described above, using the 18h enrichment broth.

Storage

Store Assurance® GDS for *E. coli* O157:H7 Tq kit components at 5 ± 3 °C. Kit expiration is provided on the product box label.

Precautions

Assurance® GDS for *E. coli* O157:H7 Tq must be used as described herein. Do not use Assurance® GDS for *E. coli* O157:H7 Tq reagents that have expired.

Safety

Assurance® GDS for *E. coli* O157:H7 Tq kit.—This product is not intended for human or veterinary use. Assurance® GDS for *E. coli* O157:H7 Tq must be used as described in the package insert. Contents of the test may be harmful if swallowed or taken internally. The user should read, understand, and follow all safety information in the instructions for the Assurance® GDS for *E. coli* O157:H7 Tq Kit. Retain the safety instructions for future reference.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with sufficient volume of a 10% bleach solution to cover tubes for a minimum of 15 min or double bag amplification tubes and dispose outside of the lab.

If contamination is suspected, moisten paper towel with bleach solution and wipe all lab benches and equipment surfaces with 10% bleach solution. Avoid spraying bleach solution directly onto surfaces. Allow bleach solution to remain on surfaces for a minimum of 15 min before wiping clean with 70% isopropyl alcohol solution.

To prepare 10% bleach solution, add 10 mL of commercially available bleach containing at least 5% sodium hypochlorite to 90 mL of deionized water. The minimum final concentration of sodium hypochlorite in the bleach solution should be 0.5%. The bleach solution is stable for 7 days from preparation. To prepare 70% isopropyl

alcohol solution, add 70 mL of pure isopropyl alcohol to 30 mL of deionized water or buy commercially available 70% isopropyl alcohol.

Assurance® GDS Rotor-Gene®.—Improper use of the Assurance® GDS Rotor-Gene® may cause personal injuries or damage to the instrument. Some components may pose a risk of personal injury due to excessive heat if improperly handled. For safe use, the instrument must only be operated by qualified laboratory personnel who have been appropriately trained. Servicing of instrument must only be performed by MilliporeSigma/ Merck KGaA Service Engineers.

Sample Enrichment.— To reduce the risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow standard laboratory safety practices, including wearing appropriate personal protective apparel and eye protection, PPE, while handling reagents and contaminated samples. Avoid contact with the contents of the enrichment media and reagent tubes after amplification. Dispose of enriched samples according to current industry standards. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state, and federal regulations.

E. coli O157:H7 Precautions—*E. coli* O157:H7 is a biosafety level-2 organism. Biological samples, such as enrichments, have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes, but is not limited to: protective eyewear, face shield, clothing/laboratory coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture based confirmatory steps. Clean the workstations and laboratory equipment with a disinfectant of choice before and after lab activities (sodium hypochlorite solution, phenol solution, quaternary ammonium solution, etc.).

Manufacturing Entity

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