Assurance® GDS MPX ID for Top STEC

NF Validation Certificate N° TRA 02/13-04/22

Part No: 71019-52 (52 tests)

General Description

Assurance® GDS MPX ID for Top STEC (MPX ID) is an automated nucleic acid amplification system for the genetic identification and confirmation of "Top Six" non-O157 Shiga toxigenic *E. coli* (STEC) in raw meat and ready-to-cook meat products, raw milk and dairy products. The Top 6 non-O157 STEC are defined as *E. coli* belonging to serogroups O103, O111, O121, O145, O26, or O45 that possess both the *eae* gene and one or both of the Shiga Toxin genes, *stx1* or *stx2*. ISO/TS 13136 (2012) targets only the Top 4 non-O157 STEC (O103, O111, O145 and O26). This NF method is validated only for identification and confirmation of these Top 4 non-O157 STEC serotypes.

The MPX ID assay utilizes a proprietary IMS-based sample preparation procedure to capture organisms belonging to 6 specific Top STEC O-serogroups (O103, O111, O121, O145, O26, and O45) prior to genetic analysis for the associated pathogenicity and O-antigen group genes. Each of the 3 unique *eae* gene sequences targeted by MPX ID in combination with one (or more) of the 6 O-antigen serogroups are highly correlated for the presence of Top 6 non-O157 STECs. Because there are 3 different *eae* gene targets and 6 different O-antigen serogroup targets, the PCR reactions have been divided between 2 amplification tubes. Each tube contains an internal control and all necessary PCR reagents. Results will automatically be combined between the 2 tubes via Assurance® GDS Rotor-Gene® software. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

The MPX ID assay is designed to follow the Assurance® GDS MPX for Top 7 STEC (Part No. 71015-100, GDS MPX) assay to detect and confirm the presence of Top 6 non-O157 STEC. However, the MPX ID assay does not detect or confirm the presence of *E. coli* O157:H7. Positive non-O157 samples from GDS MPX can be confirmed, including identification of the specific O-serogroup(s), with MPX ID. Follow SECONDARY SCREENING PROTOCOL procedure.

Additionally, MPX ID can be used to confirm isolated colonies from presumptive positive samples for Top 6 non-O157 STEC. Follow CONFIRMATION OF ISOLATED COLONIES procedure.

Kit Components

Each Assurance® GDS MPX ID for Top STEC kit contains the following:

- MPX Group 1 STEC Amplification Tubes
- MPX Group 2 STEC Amplification Tubes
- Top 6 STEC Concentration Reagent
- Resuspension Buffer Tq
- Top STEC Wash Solution

Equipment / Materials Required

Other necessary materials not provided include:

- Assurance® GDS Rotor-Gene® thermocycler
- GDS rotor and locking ring
- Laptop computer and software v2.3.103
- PickPen® device and PickPen® tips
- Vortex mixer (IKA® MS3 or equivalent)
- Adhesive film strips

Sample wells and sample well base

Resuspension plate

8-channel micropipette capable of accurately dispensing 30 µL

Adjustable micropipette capable of accurately dispensing 1.0 mL

Repeat pipette

Repeat pipette tips (0.5 mL and 10 mL)

Filter barrier micropipette tips (50 µL and 1.0 mL)

Gel cooling block(s)

Incubator capable of maintaining 41.5 ± 1 °C

Incubator capable of maintaining 37 ± 1 °C

Freezer capable of maintaining -20 ± 5 °C

Refrigerator capable of maintaining 5 ± 3 °C

SECONDARY SCREENING PROTOCOL

Sample Preparation

Please see APPENDIX A for Enrichment Methods Table.

Note: For this method, when a temperature of 41.5 °C is specified, the acceptable temperature range is 41.5 ± 1 °C.

Note: Assurance® GDS MPX ID is designed for use as a secondary assay to confirm Assurance® GDS MPX for Top 7 STEC positive results. However, the MPX ID assay does not detect or confirm *E. coli* O157:H7.

A. Test Portion Preparation

1. **Raw beef meats** - remove retained samples (enriched according to the Assurance® GDS MPX for Top 7 STEC Directions for Use) from 41.5 °C incubator after a total of 10 – 22 h of incubation. For frozen state beef, incubate for a minimum of 12 - 24 h.
2. **Raw milk and dairy products** - remove retained samples (enriched according to the Assurance® GDS MPX for Top 7 STEC Directions for Use) from 41.5 °C incubator after a total of 20 – 30 h of incubation.

Note: Total enrichment time will be based on the completion of the primary Assurance® GDS MPX for Top 7 STEC analysis.

B. Sample Extraction Protocol

Change gloves prior to handling reagents.

1. Vortex **Top 6 STEC Concentration Reagent**. Immediately transfer 40 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 **Top STEC Wash Solution** to each of 2 additional GDS sample wells (2 wells/sample) using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
3. Transfer 80 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.
4. Carefully remove the adhesive film from 1 strip of sample wells containing Top 6 STEC Concentration Reagent. Add 1.0 mL of presumptive positive enrichment to each sample well. Avoid transferring food particles. A new pipette tip must be used for each enrichment. Cover each strip of sample wells with a new adhesive film prior to adding enrichments to a new strip of wells. **Immediately return samples to incubator for use during confirmation if necessary.**

Note: Enrichments for raw milk and dairy products, after 18 h, may be retained at room temperature during sample analysis.

5. Place sealed sample wells containing the Top 6 STEC Concentration Reagent and enrichments on the vortex mixer and vortex at approximately 900 rpm for 10–20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

6. Carefully remove and discard the adhesive film from 1 strip of enrichments. Remove the corresponding adhesive film from 2 strips of sample wells containing Top STEC 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 into the first strip of enrichments. Stir tips gently for 30 s while continually moving up and down from the surface to the bottom of the wells. 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 Top STEC Wash Solution and retract PickPen® magnets to release particles into Top STEC Wash Solution.

9. Discard PickPen® tips and load a new set of tips onto the PickPen® device.

10. Extend the PickPen® magnets and insert tips into the 1st strip of sample wells containing the Top STEC Wash Solution and particles. Stir tips gently for 30 s while continually moving 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 droplets of Top STEC Wash Solution.

11. Transfer PickPen® tips to the second set of sample wells containing fresh Top STEC Wash Solution and gently swirl for 10 s (do not release particles into solution). Gently tap the PickPen® tips against the side of the sample wells to remove excess droplets of Top STEC Wash Solution.

12. Transfer particles to corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film.

13. Repeat steps (6) through (12) for all samples using new tips for each strip of enrichments.

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents.

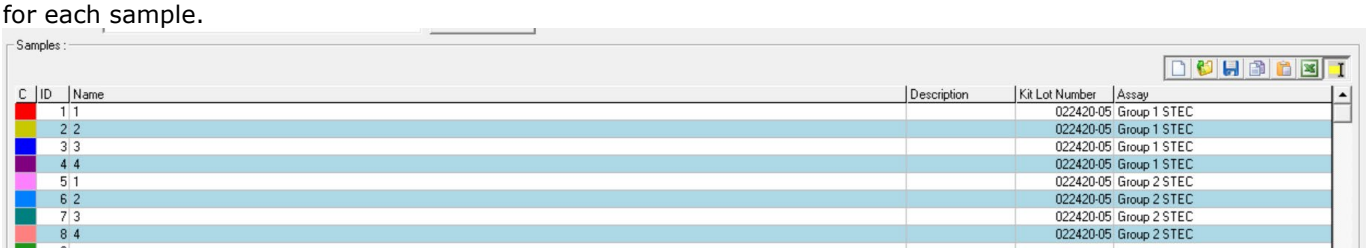
A. Preparation of Gel Cooling Blocks

1. Prior to initial use, 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 at -20 ± 5 °C.

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

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. Group 1 & 2 STEC Amplification Tubes should be named the same way when being entered in the software. If using the same name for all samples, designate the difference using numbers or letters after the name. The designation for which Amplification Tube is a Group 1 STEC or Group 2 STEC is made by choosing the correct drop-down assay for each sample.



2. Remove **Group 1 STEC Amplification Tubes** (rounded clear caps) and **Group 2 STEC Amplification Tubes** (flat blue caps) from foil pouch and place them in 2 separate columns in one frozen gel cooling block. Reseal pouch.

3. Open Amplification Tubes. Using a multi-channel pipette and filter barrier tips, briefly pipette up and down the Resuspension Buffer Tq to mix beads in resuspension plate wells. Transfer 30 µL of sample from the resuspension plate wells into each of the Amplification Tube Groups (Group 1 STEC and Group 2 STEC). Firmly press down on each Amplification Tube lid to close. Visually inspect each Amplification Tube to ensure that the cap is securely sealed.
4. Place both sets of Amplification Tubes into Assurance® GDS Rotor-Gene® in sequential order, beginning with position #1. Start Rotor-Gene® cycle. Refer to Assurance® GDS User Manual 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** or **Negative** for Top 6 non-O157 STEC or **No Amp** in the Combined Results Tab.

Positive: Sample is positive for one or more of the Top 6 non-O157 STEC, meaning *E. coli* that belong to O-serogroups O103, O111, O121, O145, O26, and O45 and contain the *eae* gene and one or both of the Shiga toxin genes, *stx1* or *stx2*. The specific serotype(s) of the Top 6 non-O157 STEC contained in sample are identified by MPX ID. The *stx* results were provided from the primary Assurance® GDS MPX for Top 7 STEC analysis.

Negative: Sample is negative for the Top 6 non-O157 STEC.

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

STEC CT Table | Combined Results

No.	C	Name	eae1 CT	eae2 CT	eae3 CT	O26 CT	O45 CT	O103 CT	O111/145 CT	O121 CT
5		1	21.65				22.60	21.82		22.09
6		2		21.14	20.97	20.96			21.86	
7		3								
8		4								

STEC CT Table | Combined Results

No.	C	Name	Top 6 STEC	O26	O45	O103	O111	O121	O145	Assay	Kit Lot Number
5		1	Positive		+	+		+		Assurance GDS MPX ID for Top STEC	022420-05
6		2	Positive	+			+		+	Assurance GDS MPX ID for Top STEC	022420-05
7		3	Negative							Assurance GDS MPX ID for Top STEC	022420-05
8		4	No Amp							Assurance GDS MPX ID for Top STEC	022420-05

Note: Assurance® GDS MPX ID for Top STEC is intended for identification and confirmation of the Top 6 non-O157 STEC serotypes; however any *eae*-positive *E. coli* isolate that also contains *stx1* and/or *stx2* would be considered a potentially pathogenic strain.

Note: Enriched samples can be stored at 5 ± 3 °C for up to 72 h prior to testing with MPX ID.

Cultural Confirmation

Following 10 – 30 h enrichment (based on food type analyzed) in mEHEC® at 41.5 °C, samples can be confirmed from the retained mEHEC® enrichment via the following. Confirm samples by ISO method or alternative confirmation.

Note: For all isolation methods, enriched samples can be stored at 5 ± 3 °C (refrigeration) for up to 72 h prior to confirmation.

A. An aliquot of the mEHEC® enrichment from Assurance® GDS MPX ID for Top STEC positive samples may be culturally confirmed for ISO Top 4 non-O157 STEC via ISO/TS 13136:2012 *Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups*.

B. Top 6 non-O157 STEC may be isolated from MPX ID positive samples by plating the Top 6 STEC Concentration Reagent which remains in the resuspension plate (step C, Test Procedure). Plate IMS beads to chromogenic agar plates. Streak plates for isolation. Incubate plates for 20–24 h at 37 ± 1 °C.

Isolation of Top 6 STEC using Concentration Reagent Remainder in Resuspension Plate

Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:

Top STEC Wash Solution
Sterile disposable 10 µL inoculating loops

1. Add 30 µL Top STEC Wash Solution, using a repeat pipette and a 0.5 mL pipette tip, to the required number of unused wells in a new resuspension plate (1 well/sample). Cover resuspension plate wells containing Wash Solution with adhesive film strips.
2. From the sample resuspension plate previously used for MPX ID analysis (step B, Test Procedure), briefly pipette up and down remainder liquid contained in the well to be plated. This will resuspend the IMS beads contained in Resuspension Buffer Tq (approximately 20 µL volume remains).
3. Remove 1 adhesive film strip from Top STEC Wash Solution resuspension plate from step 1. Transfer 15 µL suspended IMS beads to 1 resuspension plate well containing 30 µL Top STEC Wash Solution.
4. Briefly pipette up and down the Wash Solution to mix beads in well. Transfer 20 µL from the resuspension plate to first quadrant of CHROMagar™ STEC plate. Transfer additional 20 µL from the resuspension plate to first quadrant of either Rainbow® agar with Cefixime and Novobiocin (RANC) agar, ChromID® EHEC, or Tryptone Bile X-Glucuronide (TBX) agar plates. Streak beads for isolation on chromogenic plates. Incubate plates for 20–24 h at 37 ± 1 °C.

   Note: CHROMagar™ STEC and Rainbow® agar with novobiocin and cefixime plates are selective for STEC. ChromID® EHEC or Tryptone Bile X-Glucuronide (TBX) agar plates are selective for all *E. coli* (including STEC). These latter 2 plate types will need to have typical *E. coli* colonies point inoculated and screened as colony pools initially to find the putative STEC organisms, since they select for all *E. coli*. For more detail on point inoculation, see ISO 13136.

5. Confirm typical colonies by analysis of isolated colonies by Assurance® GDS MPX for Top 7 STEC and Assurance® GDS MPX ID for Top STEC, using protocol CONFIRMATION OF ISOLATED COLONIES FOR TOP STEC BY MPX ID, below.

   Note: The original MPX ID resuspension buffer plate can be stored at 5 ± 3 °C (refrigeration) for up to 48 h prior to confirmation.

C. Top 6 non-O157 STEC may be culturally isolated from MPX ID positive samples using the Top 6 STEC Concentration Reagent (included with Assurance® GDS MPX ID for Top STEC kit), which contains a mixture of IMS particles containing all Top 6 non-O157 STEC O-serogroups. This method utilizes the Assurance® GDS PickPen® device to isolate using the Top 6 STEC Concentration Reagent. Plate IMS beads to chromogenic agar plates. Streak plates for isolation. Incubate plates for 20–24 h at 37 ± 1 °C.

Isolation of Top 6 STEC using Concentration Reagent and PickPen™ device

Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:

Top 6 STEC Concentration Reagent

Top STEC Wash Solution

Sterile disposable 10 µL inoculating loops

1. Aliquot 40 µL, using a repeat pipette and a 0.5 mL pipette tip, of homogenized Top 6 STEC Concentration Reagent into the GDS sample wells (1 well/sample). Cover sample wells with adhesive film strips.
2. Add 1 mL, using a repeat pipette and a 10 mL pipette tip, of Top STEC Wash Solution into 2 sets of GDS sample wells (2 wells/sample). Cover sample wells with adhesive film strips.
3. Add 50 µL of Top STEC Wash Solution, using a repeat pipette and a 0.5 mL pipette tip, to the required number of wells in a new resuspension plate (1 well/sample). Cover resuspension plate wells containing Wash Solution with adhesive film strips.
4. Carefully remove adhesive film from 1 strip of GDS sample wells containing Concentration Reagent. Following incubation, gently mix enriched presumptive positive enrichments by hand to ensure homogeneity. Add 1 mL of the enrichment to each sample well. Avoid transferring food particles. A new pipette tip must be used for each enrichment. Seal each row of the sample wells with new adhesive film strip.
5. Place the sealed sample wells containing the Top 6 STEC Concentration Reagent and enrichments on the plate vortex mixer at approximately 900 RPM for 10-20 min.

   Note: If necessary, adjust the RPM of the vortex mixer to be certain that liquid does not contact adhesive film.
6. Carefully remove and discard the adhesive film from a strip of enrichments. Remove the corresponding adhesive film from 2 strips of sample wells containing Top STEC 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 into the first strip of enrichments. Stir tips gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen™ tips against the side of the sample wells to remove excess media droplets.
8. Transfer PickPen® tips to corresponding 1st strip of sample wells containing Top STEC Wash Solution and retract PickPen® magnets to release particles into Top STEC Wash Solution.
9. Discard PickPen® tips and load a new set of tips onto the PickPen™ device.
10. Extend the PickPen® magnets and insert tips into the strip of wells containing the Top STEC Wash Solution and particles. Stir tips gently for 30 s while continually moving 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 droplets of Top STEC Wash Solution.
11. Transfer PickPen® tips to the second set of sample wells containing fresh Top STEC Wash Solution and gently swirl for 10 s (do not release particles into solution). Gently tap the PickPen® tips against the side of the sample wells to remove excess droplets of Top STEC Wash Solution.
12. Remove 1 adhesive film strip from Top STEC Wash Solution resuspension plate from step 3. 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 Top STEC Wash Solution in resuspension plate.
13. Briefly pipette up and down the Wash Solution to mix beads. Transfer 20 µL from the resuspension plate to first quadrant of CHROMagar® STEC plates. Transfer additional 20 µL from the resuspension plate to first quadrant of either Rainbow® agar with Cefixime and Novobiocin (RANC agar), ChromID® EHEC, or Tryptone Bile X-Glucuronide (TBX) agar plates. Streak beads for isolation on chromogenic plates. Incubate plates for 20–24 h at 37 ± 1 °C.

    Note: CHROMgar STEC and Rainbow® agar with novobiocin and cefixime plates are selective for STEC. ChromID® EHEC or Tryptone Bile X-Glucuronide (TBX) agar plates are selective for all *E. coli*

(including STEC). These latter 2 plate types will need to have typical *E. coli* colonies point inoculated and screened as colony pools initially to find the putative STEC organisms, since they select for all *E. coli*. For more detail on point inoculation, see ISO 13136.

14. Confirm typical colonies by analysis of isolated colonies by Assurance® GDS MPX for Top 7 STEC and Assurance® GDS MPX ID for Top STEC assays, using protocol CONFIRMATION OF ISOLATED COLONIES FOR TOP STEC BY MPX ID, below.

Confirmation of Isolated Colonies for Top STEC by MPX ID

Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:

Top STEC Wash Solution

Resuspension Buffer Tq

Sterile disposable 1 µL inoculating loops

A. Colony Preparation

1. Transfer 500 µL of Top STEC Wash Solution, using a repeat pipette and a 10 mL pipette tip, to the appropriate number of GDS sample wells (1 well for each suspect colony).

Note: The colony tested must be well isolated. If not, re-streak for purity before continuing colony confirmation.

2. Pipet 120 µL of Resuspension Buffer Tq, using a repeat pipette and a 0.5 mL pipette tip, to the required number of wells in the resuspension plate (1 well / test).

3. Using a 1 µL sterile loop, transfer a small amount of the suspect colony to the sample well containing the Top STEC Wash Solution.

Note: Avoid heavy turbidity for the colony resuspension. It is not necessary to create obvious turbidity in the sample. Mix with the loop for about 5 s, and discard the loop.

4. Using a new sterile 1 µL loop, transfer 1 µL of the colony suspension into the prepared resuspension plate well. Stir gently with the loop.
5. Repeat steps (3) through (4) for all suspect colonies to be analyzed.
6. Proceed with Assurance® GDS MPX for Top 7 STEC and Assurance® MPX ID for Top STEC assays as specified in the directions for use [starting at step **A.** TEST PROCEDURE (AMPLIFICATION & DETECTION)].

B. Interpretation and Colony Confirmation Results

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

Positive: An isolate will be considered confirmed positive for ISO Top 4 non-O157 STEC if both of the following criteria are obtained:

1. Positive result for MPX ID: MPX ID assay identifies presence of ISO Top 4 O-serogroups: O103, O111, O145, or O26.
2. Positive result for GDS MPX: GDS MPX assay provides both the *eae* gene and *stx1* and/or *stx2* genes.

Negative: Isolate is confirmed negative if it does not meet the criteria as described above.

No Amp: Amplification did not occur. Repeat the test beginning from step **A. Colony Preparation**. If the No Amp result is repeated contact Technical Service (BioMTS@milliporesigma.com).

Note: The Assurance® GDS MPX ID for Top STEC assay will also confirm serotypes O45 and O121 STEC. These serotypes of STEC are not targeted by ISO 13136 (2012). In addition, certain strains of STEC O145 may be positive for the *eae* gene only by the Assurance® GDS MPX for Top 7 STEC results (and not Assurance® GDS MPX ID for Top STEC assay).

Storage

Store Assurance® GDS MPX ID kit components at 5 ± 3 °C.

Precautions

Assurance® GDS MPX ID for Top STEC must be used as described herein. Do not use Assurance® GDS MPX ID for Top STEC reagents that have expired. Do not use test kit beyond expiration date on the product box label.

Safety

Assurance® GDS MPX ID for Top STEC kit.—This product is not intended for human or veterinary use. Assurance® GDS MPX ID for Top STEC 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 MPX ID for Top STEC 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. Follow all applicable local, state/provincial, and/or national regulations on disposal of used Amplification Tubes. 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.

Shiga Toxigenic *E. coli* (STEC) Precautions.—STEC is a biosafety level-3 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.).

APPENDIX A – Enrichment Methods

Table 1. Sample Type and Enrichment Method for Top 6 non-O157 STEC

Please note that the sample should be taken from the retained enrichment, as described in the Assurance® GDS MPX for Top 7 STEC Directions for Use.

Food Category	Media	Sample size	Sample:Media Ratio (Media Volume)	Enrichment Time	Incubation Temperature
Raw beef meats and ready-to-cook beef meat products	mEHEC	25 g	1:10 (225 mL)	10-18 h	41.5 ± 1 °C
		375 g	1:5 (1500 mL)	12-20 h	
Seasoned beef	mEHEC	25 g	1:10 (225 mL)	10-18 h	
Raw milk and dairy products (except raw milk cheese)	mEHEC	25 g	1:10 (225 mL)	20-26 h	
Raw milk cheese	mEHEC	25 g	1:10 (225 mL)	22-30 h	

APPENDIX B

Rainbow® Agar with Novobiocin and Cefixime (RANC)

Ingredient	Qty/ Liter	Source
Rainbow® agar O157 base	60 g	Biolog Cat 80102
Novobiocin	5 mg	Sigma N1628
Cefixime	5 µg	Dynal Cat 740.01

Preparation of RANC Plates:

1. Add 60 g Rainbow® Agar Base to 1000 mL deionized water. Heat with frequent agitation and boil for 1 minto completely dissolve the powder.
2. Autoclave at 121 °C for 10 min.
3. Cool media to 45–50 °C.
4. Add the following antibiotic solutions and mix well:
   a. 2.5 mL novobiocin solution/L of media.
   b. 375 µL cefixime solution/L of media.
5. Pour agar plates (~20 mL per plate).

Preparation of Antibiotic Solutions:

Novobiocin solution**:** Add 0.1 g novobiocin to 50 mL sterile water (2 mg/mL).

Cefixime Solution: Add 7.5 mL sterile water to 1 mg cefixime (0.133 µg/µL).

NF Validation certificate granted by AFNOR Certification for Assurance® GDS MPX ID for Top STEC as an alternative method of analysis for all food products and industrial production environmental samples in relation to the reference method described in the ISO EN 13136 international standard in accordance with EN ISO 16140-2 (2016). For more information about the NF VALIDATION certification, please refer to the certificate available at http://nf-validation.afnor.org/en

TRA 02/13-04/22

ALTERNATIVE METHODS OF ANALYSIS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

Manufacturing Entity

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