

Assurance® GDS MPX Top 7 STEC

NF VALIDATION Certification TRA 02/13-04/22

Part No: 71015-100 (100 tests)

General Description

Assurance® GDS MPX for Top 7 STEC is an automated nucleic acid amplification system for the detection of *E. coli* O157:H7 and the "Top Six" non-O157 Shiga Toxigenic *E. coli* (STEC) in raw meat, ready-to-cook (RTC) meat products, raw meat poultry, ready-to-cook (RTC) meat poultry products, ready-to-eat (RTE) meat poultry products, ready-to-reheat (RTRH) meat poultry products, raw milk and dairy products, environmental samples. The Top Six 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 at least one of the Shiga toxin genes, *stx1* or *stx2*. Assurance® GDS MPX for Top 7 STEC utilizes a proprietary IMS-based sample preparation procedure to capture organisms belonging to 7 specific Top STEC O-serogroups (O103, O111, O121, O145, O26, O45, and O157) prior to genetic analysis for the associated pathogenicity genes. ISO/TS 13136 (2012) targets only the Top 5 STEC (O157, O103, O111, O145, and O26). This NF method is validated only for the detection and confirmation of the Top 5 STEC serotypes.

This STEC detection method is based on the workflow described in ISO/TS 13136:2012 and includes two screening steps from enriched samples:

1. Detection of the *eae* and *stx* genes of organisms belonging to the Top STEC using Assurance® GDS MPX for Top 7 STEC (Lit. No. 20764278)
2. Detection of the O-groups using Assurance® GDS MPX ID for Top STEC (Lit. No. 20764279) and/or Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq (Lit. No. 20764281)

Positive screening results must be confirmed by plating on selective agar and testing characteristic colonies (Lit. No. 20764279 and 20764281).

Kit Components

Each Assurance® GDS MPX Top 7 STEC kit contains the following:

- MPX Top STEC Amplification Tubes
- Top 7 STEC Concentration Reagent
- Resuspension Buffer Tq
- Top STEC Wash Solution

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 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
- Stomacher® paddle homogenizer or equivalent

Stomacher®-type bags with filter or equivalent
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

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

Enrichment Media Preparation

- A. For 25 g sample, pre-warm 225 mL sterile deionized water at 41.5 ± 1 °C overnight. On day of use, aseptically transfer 7.1 g of mEHEC® media into the pre-warmed sterile water. Gently mix to rehydrate the media. Use prepared broth within 6 h.
- B. For 375 g sample, pre-warm 1500 mL sterile deionized water at 41.5 ± 1 °C overnight. On day of use, aseptically transfer 47.3 g of mEHEC® media into the pre-warmed sterile water. Gently mix to rehydrate the media. 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 rehydrate the media, dispense into desired volume and autoclave at 121 °C for 15 min. Broth must be pre-warmed to 41.5 ± 1 °C overnight prior to sample addition.

Sample Preparation

Application in raw meat, RTC meat, raw poultry, RTC poultry, RTRH poultry, RTE poultry, raw milk and dairy products (up to 25 g) or raw beef meat (up to 375 g) or environmental samples (25 g, mL or sampling device).

A. Test Portion Preparation & Enrichment

Please see APPENDIX A for Enrichment Methods Table

Note: Follow instructions of EN ISO 6887 standards (addition of Tween-80 (10 g/L) for matrices >20% fat).

1. **Raw meat and RTC meat up to 25 g** – For 25 g test portion, add sample into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. Homogenize or mix sample and incubate for 6–24 h at 41.5 ± 1 °C.
2. **Raw beef meat up to 375 g** – For 375 g test portion, add sample into 1500 mL pre-warmed (41.5 ± 1 °C) mEHEC® media without following EN ISO 6887 standards (no addition of Tween-80). Homogenize or mix sample and incubate for 10–18 h at 41.5 ± 1 °C. If alternate test portion sizes are analyzed, proportionately adjust the volume of media to maintain 1:5 ratio.
3. **Raw meat poultry, RTC, RTRH and RTE meat poultry up to 25 g** – For 25 g test portion, add sample into 225 mL pre-warmed (37 ± 1 °C) mEHEC® media. Homogenize or mix sample and incubate for 8–24 h at 41.5 ± 1 °C.
4. **Raw milk and dairy products up to 25 g** – For 25 g test portion, add sample into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. Homogenize or mix sample and incubate for 10–24 h at 41.5 ± 1 °C **or** for 18 – 24 h at 41.5 °C without following EN ISO 6887 standards (no addition of Tween-80).

5. **Environmental samples up to 25 g, mL or sampling device** – Aseptically weigh 25 g sweepings or 25 mL process water into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC[®] media. For environmental monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of mEHEC[®] media, respectively. Incubate for 10–24 h at 41.5 ± 1 °C.

Note: Contact your local technical service for recommended procedures for testing alternate sample types or sizes.

Note: Enriched samples can be stored at 2–8 °C (refrigeration) for up to 72 h prior to testing with Assurance[®] GDS MPX Top 7 STEC (not applicable for short enrichment protocols).

B. Sample Extraction Protocol

Change gloves prior to handling reagents.

All foods except raw milk cheese enriched without following EN ISO 6887 standards

1. Vortex **Top 7 STEC 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 **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 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 resuspension plate with adhesive film strips.
4. Carefully remove the adhesive film from 1 strip of sample wells containing Top 7 STEC Concentration Reagent. Mix incubated sample enrichments. Add 1.0 mL of enrichment 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 enrichments to the next strip of wells. **Immediately return samples to incubator for use during confirmation, if necessary.**
5. Place sealed sample wells containing the Top 7 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 and from resuspension plate.
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 strip of enrichments. 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 media droplets.
8. Transfer PickPen[™] tips to corresponding first set 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 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). 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.

PROCEED TO TEST PROCEDURE SECTION

Raw milk cheese enriched without following EN ISO 6887 standards

1. Vortex **Top 7 STEC 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 **Top STEC Wash Solution** to each of 2 additional sample wells (2 wells/sample) using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
3. Dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well / sample) using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
4. Transfer 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 resuspension plate with adhesive film strips.
5. Carefully remove the adhesive film from 1 strip of sample wells containing Top 7 STEC Concentration Reagent. Mix incubated enrichments. Add 1.0 mL of incubated enrichment 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 of wells. **Immediately return samples to incubator for use during confirmation, if necessary.**

Note: Enrichments for raw cheese, after 18 h, may be retained at room temperature during sample analysis.

6. Place sealed sample wells containing the Top 7 STEC Concentration Reagent and enrichments on the vortex mixer and agitate at approximately 900 rpm for 10–20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.
7. Carefully remove and discard the adhesive film from 1 strip of enrichments. Remove the corresponding adhesive film from 1 strip of sample well containing Top STEC Wash Solution. Also remove the corresponding adhesive film for strip of sample wells containing BHI.
8. 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 enrichments.

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 media droplets.
9. Transfer PickPen™ tips to set of sample wells containing fresh Top STEC 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 Top STEC Wash Solution.
10. Transfer PickPen™ tips to corresponding sample wells containing BHI and retract PickPen™ magnets to release particles into BHI.
11. Cover each strip of samples wells containing BHI with an adhesive film prior to adding additional samples to a new strip of sample wells. Incubate BHI sample wells containing particles for 2 – 4 h at 37 ± 1 °C. Repeat steps (7) – (11) for all samples.
12. After incubation, remove the corresponding adhesive film from remaining strip of sample well containing Top STEC Wash Solution. Carefully remove and discard the adhesive film from strip of BHI samples. Extend the PickPen™ magnets and insert tips into the strip of sample wells containing the BHI 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 BHI.
13. 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). Tap the PickPen™ tips against the side of the sample wells to remove excess droplets of Top STEC Wash Solution.

14. 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.
15. Repeat steps (12) through (14) for all samples using new PickPen™ tips for each strip of enrichments.

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents.

A. Preparation of Gel Cooling Block

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**.
2. Remove MPX Top STEC Amplification Tubes from foil pouch and place them in the frozen gel cooling block. Reseal pouch. Carefully check that the white pellet is at the bottom of the Amplification Tubes.
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 Amplification Tube. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.
4. Place 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 STEC, **Positive** or **Negative** for *E. coli* O157:H7, or **No Amp**. The individual gene results (*eae*, *stx1*, *stx2*) are also presented.

Top STEC (*eae/stx*) Results:

Positive: Samples are presumptive positive for Top STEC, meaning they are *E. coli* that belong to O-serogroups O103, O111, O121, O145, O26, O45, and O157 and contain the *eae* gene and one or both of the Shiga toxin genes, *stx1* or *stx2*.

Negative: Samples are negative for Top 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 your local technical service.

No.	Name	Top STEC Result	eae Result	stx1 result	stx2 result	Assay	Kit lot
1	Sample 1	Positive	+	+	+	Top STEC MPX	abc123
2	Sample 2	Positive	+	+	-	Top STEC MPX	abc123
3	Sample 3	Positive	+	-	+	Top STEC MPX	abc123
4	Sample 4	Negative	+	-	-	Top STEC MPX	abc123
5	Sample 5	Negative	-	+	+	Top STEC MPX	abc123
6	Sample 6	Negative	-	+	-	Top STEC MPX	abc123
7	Sample 7	Negative	-	-	+	Top STEC MPX	abc123
8	Sample 8	Negative	-	-	-	Top STEC MPX	abc123
9	Sample 9	No Amp	-	-	-	Top STEC MPX	abc123

E. coli O157:H7 Results:

Positive: Samples are presumptive positive for *E. coli* O157:H7. Certain strains of STEC belonging to the O145 serogroup may also be indicated as 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 **B. Sample Extraction Protocol**. If the No Amp result is repeated contact your local technical service.

No.	Name	E. coli O157:H7 Result	Assay	Kit lot
1	Sample 1	Positive	Top STEC MPX	abc123
2	Sample 2	Positive	Top STEC MPX	abc123
3	Sample 3	Positive	Top STEC MPX	abc123
4	Sample 4	Negative	Top STEC MPX	abc123
5	Sample 5	Negative	Top STEC MPX	abc123
6	Sample 6	Negative	Top STEC MPX	abc123
7	Sample 7	Negative	Top STEC MPX	abc123
8	Sample 8	Negative	Top STEC MPX	abc123
9	Sample 9	No Amp	Top STEC MPX	abc123

Note: Assurance® GDS MPX for Top 7 STEC is intended for detection of the Top 7 STEC serotypes; however, any *eae*-positive *E. coli* isolate that also contains *stx1* and/or *stx2* would be considered a potentially pathogenic strain.

Confirmation

In the context of NF VALIDATION, all samples identified as positive by Assurance® GDS MPX Top 7 STEC must be confirmed by the following tests:

- Top 6 non-O157 STEC, Top 6 non-O157 STEC are identified by a combination of a positive result from TOP STEC channel and a negative result from O157 channel. An aliquot of the mEHEC® enrichment from GDS MPX for Top 7 STEC positive samples may be confirmed for Top 6 STEC via secondary analysis by the MPX ID assay.

Note: The NF method will be validated only for the confirmation of the Top 4 non-O157 STEC (O111, O26, O103 and O145) serotypes listed in ISO/TS 13136 (2012).

- E. coli* O157:H7, an aliquot of the mEHEC® enrichment from GDS MPX for Top 7 STEC positive samples may be confirmed for *E. coli* O157:H7 via secondary analysis by GDS EHEC ID and MPX ID assays, to distinguish between O157 and O145, as needed. Certain strains of STEC belonging to the O145 serogroup may also be indicated as positive for *E. coli* O157:H7.

Note: In the event of discordant results (positive presumptive results with the alternative method, non-confirmed by one the means above), the laboratory must follow the necessary steps to ensure validity of the result obtained.

Storage

Store Assurance® GDS MPX for Top 7 STEC kit components at 5 ± 3 °C. Kit expiration is provided on the product box label.

Precautions

Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).

Assurance® GDS MPX for Top 7 STEC kit must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

Do not use Assurance® GDS MPX for Top 7 STEC reagents that have expired. Do not use test kit beyond expiration date on the product box label.

Safety

Assurance® GDS MPX for Top 7 STEC kit.—This product is not intended for human or veterinary use. Assurance® GDS MPX for Top 7 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 for MPX Top 7 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 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 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 are 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 7 STEC

Food Category	Media	Sample size	Sample:Media Ratio (Media Volume)	Enrichment Time	Enrichment Temperature (media pre-warm)
No BHI subculture					
Raw meat and RTC meat*	mEHEC®	Up to 25 g	1:10 (225 mL)	6–24 h	41.5 ± 1 °C (41.5 ± 1 °C)
Raw beef meat		Up to 375 g	1:5 (1500 mL)	10–18 h	41.5 ± 1 °C (41.5 ± 1 °C)
Raw meat poultry RTC, RTRH and RTE meat poultry*		Up to 25 g	1:10 (225 mL)	8–24 h	41.5 ± 1 °C (37 ± 1 °C)
Raw milk and dairy products*		Up to 25 g	1:10 (225 mL)	10–24 h	41.5 ± 1 °C (41.5 ± 1 °C)
Raw milk and dairy products (except raw milk cheese)		Up to 25 g	1:10 (225 mL)	18–24 h	41.5 ± 1 °C (41.5 ± 1 °C)
Environmental samples*		Up to 25 g or mL or sampling device	1:10	10–24 h	41.5 ± 1 °C (41.5 ± 1 °C)
With BHI subculture					
Raw milk cheese	mEHEC®	Up to 25 g	1:10 (225 mL)	18–24 h + 2–4 h BHI	41.5 ± 1 °C (41.5 ± 1 °C)

* For these protocols, follow EN ISO 6887 standards (addition of Tween-80 (10 g/L) for matrices >20% fat).

NF Validation certificate granted by AFNOR Certification for Assurance® GDS MPX for Top 7 STEC as an alternative analytical method for the detection of Top 7 STEC in raw meat, ready-to-cook (RTC) meat products, raw meat poultry, ready-to-cook (RTC) meat poultry products, ready-to-eat (RTE) meat poultry products, ready-to-reheat (RTRH) meat poultry products, raw milk and dairy products, and 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 end of validity of the NF VALIDATION certification, please refer to the certificate TRA 02/13-04/22 available on the website <http://nf-validation.afnor.org/en>.



TRA 02/13-04/22

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

<http://nf-validation.afnor.org/en>

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