FIELD OF APPLICATION
Selective chromogenic medium for the detection, isolation and presumptive identification of *Escherichia coli* O157:H7 in products for use in human food and environmental samples.

PRINCIPLES
The RAPID’E.coli O157:H7 medium is a selective medium combining chromogenic substrates and biochemical indicators. This combination provides direct presumptive identification of *E.coli* O157:H7, including atypical strains, among the interfering flora on the basis of the specific metabolic and enzymatic profiles observed.

The selectivity of the medium is increased by adding selective agents: novobiocin (10 mg/l) and potassium tellurite (0.8 mg/l).

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol
The RAPID’E. coli O157:H7 method has been certified NF VALIDATION as an alternative to the reference standard, NF EN ISO 16654, according to the ISO 16140 protocol, for the detection of *Escherichia coli* O157:H7 for all human food products and for environmental samples.

STANDARD REFERENCES
NF EN ISO 16654 (July 2001)
Food microbiology – Horizontal method for the detection of *Escherichia coli* O157

PRESENTATION
Dehydrated
- 100g code 356-4748
- Novobiocin (one 1-g bottle) code 356-4610

PRESENTATION/VALIDITY/LOT
- Dehydrated: 2 - 8°C, carefully closed bottle
- Expiry date and lot number are indicated on the packaging.

THEORETICAL FORMULA
Enrichment mixture 58 g
Selective agents 6.25 g
Chromogenic mixture 0.75 g
Agar 15 g
Distilled water qsp 1,000 mL
final pH (25°C) = 6.9 +/- 0.2

OTHER PRODUCT(S) REQUIRED NOT SUPPLIED
- mTSB broth:
  Ready to use (with novobiocin):
  6 x 225-ml bottles (ex. code 355-5426)
  Dehydrated (base): 500 g (ex. code 356-4426)
- Novobiocin (ex. One 1-g bottle, code 356-4610)
- Potassium tellurite

See the corresponding Technical Data Sheet(s)

EQUIPMENT REQUIRED, NOT SUPPLIED
(non-exhaustive list)
- Scales
- Stomacher bags with filter
- Grinder
- Stirrer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 mL)
- Filter cones for micropipettes (100(0)µL)
- Inoculating loops
- Sterile Pasteur Pipettes
- Water bath at ± 1°C
- Oven or thermostated unit at ± 1°C precision
- Immunoconcentration beads for *E. coli* O157 and magnetic rack
RAPID’E.coli O157:H7/Agar

PREPARATION OF THE DEHYDRATED MEDIUM
“Always shake before each use”

Dissolve 80 g of powder in 1 liter of distilled water. Mix to obtain a homogenous suspension. Adjust the pH to 6.9 +/-0.2 at 25°C using either NaOH or HCl 1 N. Bring to a boil until complete dissolution. Avoid overheating. DO NOT AUTOCLAVE. Cool to 45-50°C in the water bath. Sterilely add the novobiocin (code 356-4610) (qsp 10 mg/L in the complete medium) and the potassium tellurite (qsp 0.8 mg/L in the complete medium).

Mix and distribute in the sterile Petri dishes. Let dry overnight at room temperature. Final pH: 6.9 +/- 0.2 at 25°C

Novobiocin supplement: dissolve 100 mg of novobiocin (code 356-4610) in 1 ml of sterile distilled water. Sterilize by filtering through a 0.2-µm absolute filter and using a disposable syringe. This novobiocin solution can be stored for 1 month at 4°C in an opaque bottle. Reconstitution rate: 80 g/L

100 g of powder can be used to make 1.25 liters of medium

STANDARDIZED METHOD:
• Preparation of the samples / Enrichment
To be performed according to the standards of the product in question.

• Immunoseparation
Strictly follow the supplier’s recommendations for the immunoseparation protocol.

• Isolation and Incubation
Take 50 µl of beads washed and placed in suspension after immunoseparation with a sterile micropipette and perform isolation following the conventional techniques on RAPID’E.coli O157:H7 agar, in parallel with a dish of CT-SMAC. Incubate at 37°C ± 1°C.

ALTERNATIVE METHOD:
• Preparation of the sample / Selective enrichment
- Culture η g or η ml of sample in 9 x η ml of mTSB broth + novobiocin (20 mg/l).
- Incubate at 41.5°C for 16-24 hours.

• Immunoseparation
- Use a system of paramagnetic beads coated with specific antibodies for capturing E.coli O157. Carefully follow the supplier’s recommendations for the immunoseparation protocol.

• Isolation and Incubation
- Take 50 µl of beads washed and placed in suspension after immunoseparation with a micropipette with sterile cones and perform isolation in streaks following the conventional techniques on RAPID’E. coli O157:H7 agar. Incubate for 24h ± 2h at 37°C ± 1°C.

Note: In the context of NF VALIDATION mark, no samples of over 25 g were tested.

READING AND CONFIRMATION
• Reading
Read after incubation for 24h ± 2h. Typical Escherichia coli O157:H7 (sorbitol – and β−glucuronidase –) present characteristic bright, bulging colonies measuring 1 to 2 mm, dark blue to black in color with a slight black precipitate around the edges of the colony. Atypical β−glucuronidase + Escherichia coli O157:H7 form colonies of the same type. Strains of atypical sorbitol + Escherichia coli O157:H7 are also detected. These colonies will have a blue to turquoise color with a weak black precipitate around the edges of the colony.

Note: The agar will turn completely red in the presence of pure strains of E.coli O157:H7. On agar with a mixture of strains, typical Escherichia coli O157:H7 give dark blue to black colonies with a slight black precipitate around the edges of the colony, sometimes combined with a red halo.

• Confirmation of characteristic colonies
In the context of NF VALIDATION Mark, positive results must be confirmed in one of the following way:

1-Using the conventional tests described in the standardised CEN or ISO reference methods (with purification step) or using validated nucleic probes, for example: iQ-Check™ E.coli O157:H7 PCR method (code 357-8114) using isolated colonies (with or without purification step).
2. Using of latex tests for O157 and H7 starting with 1 to 3 isolated colonies. An isolation step must be performed in case of confirmation with 2 latex tests.

Note:
- When using the latex tests, carefully follow the manufacturer’s instructions and recommendations for use.
- For characteristic colonies giving a positive O157 latex test and a negative H7 latex test, the laboratory will have to apply the appropriate measures to ensure the validity of the results obtained.

3. The use of any other NF VALIDATION certified method with a different principle than that of RAPID’E.coli O157:H7. The validated protocol for this method will have to be followed in whole, except for cases with preliminary steps common to both methods.

- In case of discordant results (positive with the alternative method, not confirmed with the tests described above, and especially for Latex tests), the laboratory will have to apply the appropriate measures to ensure the validity of the results obtained.

PRECAUTIONS FOR USE
- Performing the immunoseparation step requires sufficient training and regular practice of the technique. Following these precautions for use is a prerequisite for obtaining valid, reliable results.
- When applying immunoseparation, viscous or fatty samples may cause interference in the magnetic bead capture (low retrieval, reduced specificity of the antibody action). See the suppliers’ technical solutions for handling such samples.
- Reading the latex tests may require prior training, notably for interpreting the agglutination of the H7 flagellar antigen, which can be very fine.
- The precautions for use relative to the handling of potentially contaminated products in a microbiology laboratory must be followed.
- Before using the boxes of RAPID’E.coli O157:H7, let them dry, in compliance with the EN ISO 11133-1 standard, at 25°C-50°C until the droplets disappear from the surface of the medium. Avoid prolonged drying, however, as this could alter the performances of the medium.
- Follow Good Laboratory Practices (EN ISO 7218)

QUALITY CONTROL
All products manufactured and sold by Bio-Rad are placed under a quality assurance system from reception of the raw materials to the sale of the finished products. Each lot of finished products undergoes quality control and cannot be sold unless it complies with the acceptance criteria. Documentation relative to the production and inspection of each lot is archived.

QUALITY AND PERFORMANCES OF THE TEST

<table>
<thead>
<tr>
<th>MICRO-ORGANISMS</th>
<th>Appearance of the colonies after 24 hours at 37 °C</th>
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</thead>
<tbody>
<tr>
<td>Escherichia coli O157:H7 ATCC 700728</td>
<td>(+)</td>
</tr>
<tr>
<td>Escherichia coli O157:H7 ATCC 43888</td>
<td>(+)</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>partial to inhibition</td>
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<tr>
<td>Staphylococcus aureus ATCC 6538</td>
<td>-</td>
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</table>

KEY WORDS
RAPID’E. coli O157:H7 / Escherichia coli / Escherichia coli O157 / Escherichia coli O157:H7 / Detection / Food Products / Environment / Chromogenic / Medium.