

**RAPID' *Enterobacteriaceae* / Agar****355-4012  
356-4004****DEFINITION**

Medium used for the detection and enumeration of *Enterobacteriaceae* in 24h without confirmation, in food products, for human and animal, and environmental samples.

**NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol**

The RAPID' *Enterobacteriaceae* method has been certified NF VALIDATION as alternative to reference method ISO 21528-2 (12/2004), according to the ISO 16140 protocol, for the **enumeration of *Enterobacteriaceae*** in all food products for human and animal consumption and in environmental samples.



BRD: 07/24 - 11/13  
ALTERNATIVE ANALYTICAL METHODS FOR  
AGRIBUSINESS  
Certified by AFNOR Certification  
[www.afnor-validation.com](http://www.afnor-validation.com)

**PRINCIPLE**

The principle of the RAPID' *Enterobacteriaceae* medium relies on the ability of *Enterobacteriaceae* to ferment glucose.

Due to the simultaneous presence of crystal violet and bile salts, the medium inhibits Gram positive bacteria and some Gram negative bacteria.

The combination of color indicators allows a high level of contrast of *Enterobacteriaceae* colonies which appear as red on a clear grey medium.

**PRESENTATION**

- **Ready-to-use**  
200 ml x 6 bottles **code 355-4012**
- **Dehydrated**  
500 g **code 356-4004**

**STORAGE AND SHELF LIFE**

- Ready-to-use: + 2 - 8 °C.
- Dehydrated: + 15 - 25 °C, in carefully sealed bottles in a cool dry place.
- Expiration date and batch number are shown on the package.

**THEORETICAL FORMULA**

Nutritive mix	17.3 g
Glucose	9.0 g
Selective agent	0.7 g
Color indicators	70 mg
Agar	11.0 g
Distilled water	1000 ml
pH (25°C) final = 7,4 ± 0,2	

**OTHER PRODUCTS REQUIRED (NOT SUPPLIED)**

- Distilled water

**EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)**

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 125 ml Pyrex bottles
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 ml, etc)
- Water-bath
- Thermostatically-controlled incubator
- All usual laboratory equipment

**PREPARATION OF DEHYDRATED MEDIUM Always shake before use.**

Dissolve 38 g of powder in 1L of distilled water. Wait for 5 min, and then mix thoroughly until a homogenous suspension is obtained. Heat gently swirling frequently, and then bring to boiling point until completely dissolved.

**DO NOT AUTOCLAVE.**

This medium can be used directly after the preparation, or dispensed in closed bottles, and stored in dry cool place (check the sterility of the medium before use)

**Reconstitution ratio: 38 g/L.  
500 g of powder makes 13.15L of medium.**

**PROTOCOL**

• **Preparation of samples**  
According to the standards or recommendations applicable to the product concerned.

• **Pour-plate method:**

- Pour 1 ml of the sample or its decimal dilutions in single layer (Ø 90 mm plates).
- Pour the melted medium (44 - 47 °C) and homogenize.

# RAPID' *Enterobacteriaceae* / Agar

Note: A second layer can be poured (approximately 2 mm thick) in case of high level of contamination.

## • Surface inoculation:

- Spread out 0.1 ml of the sample, and/or its decimal dilutions if necessary, on 1 Petri dish (Ø 90 mm plates).

Note: If it is necessary to estimate small numbers, for some products, spread 1 ml of stock solution over 3 dishes of Ø 90 mm (~ 0.33 ml/dish) or over 1 dish of Ø 140 mm (refer to EN ISO 7218 standard)

## • Incubation

- 37 ± 1°C for 24 ± 2 h.  
- Alternatively, a temperature of 30°C or 35°C can be chosen when the enumeration is conducted for specific *Enterobacteriaceae* (e.g. psychrotrophic *Enterobacteriaceae*)

Note : After the incubation, the Petri dishes may be stored in a refrigerator (5 ± 3°C) for 72h with the surface inoculation protocol.

## READING AND INTERPRETATION

After 24h incubation, enumerate typical *Enterobacteriaceae*.

The *Enterobacteriaceae* form red colonies (Glucose-positive) with a diameter equal to or exceeding 0.5 mm, with or without a zone of precipitation.

The reading can be also performed with the Scan 1200® (automatic colony counter). Select "Rapid Entero" parameter and follow the instructions in order to enumerate the colonies.

## PRECAUTIONS

### - Do not autoclave the media

- It is preferable to use a double-layered medium in matrices containing abundant mesophilic flora. The aim of the second layer is to limit invasion of the surface, which can interfere with reading.

- In the context of the NF VALIDATION mark spiral-plate system has not been tested.

- In the context of the NF VALIDATION mark, Scan 1200® software version "V 6.3.7 kernel Biorad 1.2" has been used.

- Refer to Scan 1200® user manual for any question regarding the use of colony counter.

- The median bias was 0.23 log CFU/g using the surface inoculation method with the automatic colony counter, during the validation.

- End of NF VALIDATION: please see the certificate BRD: 07/24–11/13. This certificate is available from Bio-Rad representative or AFNOR Certification.

- Comply with Good Laboratory Practice (EN ISO 7218)

## PERFORMANCES

The growth performances of the media are verified with the following strains:

STRAINS	Result after 24h at 37°C		
	Glucose fermentation	Diameter	PR*
<b>Productivity test</b>			
<i>Escherichia coli</i> ATCC 25922	Positive Pink to red colonies with or without precipitation halo	≥ 0.5 mm	≥ 0.5
<i>Salmonella</i> <i>Typhimurium</i> ATCC 14028	Positive Pink to red colonies with or without precipitation halo	≥ 0.5 mm	≥ 0.5
<b>Selectivity</b>			
<i>Enterococcus faecalis</i> ATCC 19433	No growth		

\* PR = Total colony count obtained on 2 plates of VRBG/total colony count on 2 plates of TCS agar.

## QUALITY CONTROL

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from reception of raw materials through to marketing of the finished products.

Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and quality control of each batch is kept on file.

## KEY WORDS

VRBG / *Enterobacteriaceae* / Food products / Detection / Enumeration / Crystal Violet / Bile salts / Glucose / Fermentation / MPN / Medium.