

CONTACT VRBG + NEUTRALIZERS

ENUMERATION AND CONFIRMATION OF *ENTEROBACTERIACEAE*

1 INTENDED USE

VRBG agar (Violet Red Bile Glucose agar) with neutralizers is used for the detection and enumeration of Enterobacteriaceae, but also for the detection of Gram-negative and bile salt resistant bacteria. This media is used for critical control points in industry (e.g. protected areas, microbiological monitoring programmes for surfaces and industrial environments).

2 PRINCIPLES

The media forms a convex meniscus that allows direct application of the agar to the control areas, whether on walls, floors, utensils, or staff. The media contains several neutralizers that inhibit any disinfectant residues on the surfaces to be tested, in order to assess the levels of contamination before and after disinfection of the food chain environment.

The neutralizers are selected to inactivate disinfectant residues that may be present on the surfaces, such as aldehydes and phenols, quaternary ammoniums, oxidising compounds.

The simultaneous presence of crystal violet and bile salts ensures the inhibition of Gram-positive bacteria.

The degradation of glucose to acid is revealed by the red colour of the pH indicator, neutral red.

3 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of media, **with neutralizers**:

| | |
|---|---------|
| - Enzymatic digest of animal tissue | 7,0 g |
| - Autolytic yeast extract | 3,0 g |
| - Glucose | 10,0 g |
| - Bile salts | 1,5 g |
| - Sodium chloride..... | 5,0 g |
| - Neutral red..... | 30,0 mg |
| - Crystal violet..... | 2,0 mg |
| - neutralizers mixture | 7,2 g |
| - Bacteriological agar..... | 13,0 g |

pH of ready-to-use media at 25 °C: 7,4 ± 0,2.

4 INSTRUCTIONS OF USE

- Use the culture media at room temperature and on a dry surface.
- Open the plate and apply the agar directly to the surface to be tested, make sure to maintain a uniform pressure over time (e.g. 500g for 10s according to NF EN ISO 18593). Then close the plate and keep the agar at 1 to 8°C in a suitable transport container and incubate within 48 hours.
- Clean the sample surface to remove any traces of nutrients, moisture and chemical or physical elements resulting from the application of the agar.
- Incubate at 37°C for 24 ± 2 hours, according to NF EN ISO 21528-1 or according to used standard.

✓ **Incubation :**
24 ± 2h at 37 ± 1°C

NOTES :

Alternatively, the temperature of 30°C may be chosen when the enumeration of mesophilic Enterobacteriaceae is undertaken as part of the sanitary control of a technological process.

It is recommended that a control of the efficiency of the mixture of neutralizers present in the media be carried out in relation to the disinfectant product used, given the diversity of antiseptics existing on the market.

5 RESULTS

Enterobacteriaceae show pink-red to purplish colonies, with or without a purple halo of precipitated bile salts.

Proceed with the counting of the colonies. The grid at the bottom of the plates facilitates counting.

See **ANNEX 1: PHOTO SUPPORT**.

Divide the number of characteristic colonies by the area of the sampled surface and deduce the number of colony-forming units (CFU) per square centimetre of surface.

6 QUALITY CONTROL

Typical culture response after 24 hours of incubation at 37°C (NF EN ISO 11133):

| Microorganisms | | Growth (Productivity Ratio : P_R) | Characteristics |
|-------------------------------|------------|--|---|
| <i>Salmonella</i> Typhimurium | WDCM 00031 | $P_R \geq 50 \%$ | Pink-red to purplish colonies, with a violet halo |
| <i>Escherichia coli</i> | WDCM 00012 | $P_R \geq 50 \%$ | Pink-red to purplish colonies, with a violet halo |
| <i>Enterococcus faecalis</i> | WDCM 00087 | Inhibited | - |

7 STORAGE / SHELF LIFE

Ready-to-use media: 2-8 °C

Expiry dates are indicated on the labels.

8 PACKAGING

Pre-poured media in plates (Ø 65 mm):

20 plates..... BM20708

9 BIBLIOGRAPHY

NF V08-054. April 2009. Microbiology of food. Enumeration of presumptive Enterobacteriaceae by counting of colonies obtained at 30°C or 37°C

NF EN ISO 11133. July 2014. Microbiology of food, feed and water - Preparation, production, storage and performance testing of culture media (Drawing 2 (2016-01-01)).

NF EN ISO 21528-1. July 2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 1: detection of Enterobacteriaceae.

NF EN ISO 21528-2. July 2017. Microbiology of the food chain. Horizontal methods for the detection and enumeration of Enterobacteriaceae. Part 2: Colony counting method.

NF EN ISO 18593. July 2018. Microbiology of the food chain-Horizontal methods for surface sampling.

10 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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CONTACT VRBG + NEUTRALIZERS

Enumeration and Confirmation of *Enterobacteriaceae*

Reading:

Growth obtained after 24 hours of incubation at 37 °C.

