

# Violet Red Bile Dextrose Agar (VRBD)

Reference Number 0185e, 185e, 245e, 533200

## Intended Use

VRBD Agar (violet red bile dextrose agar) is used for selective isolation of *Enterobacteriaceae* or bile tolerant gram-negative bacteria from non-sterile pharmaceutical products, foodstuffs and other sample material.

The formulation of the medium is prepared according to the recommendations of harmonised methods of European Pharmacopoeia (EP 5.6) and United States Pharmacopoeia (USP 30 NF 25) as well as ISO 21528-1 und -2.

VRBD Agar is available as **LI**-medium within 90 mm plates (heipha reference 0185e), as standard medium in 90 mm plates (ref. 185e), as **RT**-contact agar in 55 mm plates (ref. 245e) and in bottles with a filling volume of 200 ml for the pour plate method (ref. 533200).

## Typical Composition per litre

Pancreatic Digest of Gelatin	7 g
Yeast Extract	3 g
Bile Salts	1.5 g
NaCl	5 g
Glucose x H <sub>2</sub> O	10 g
Neutral Red	30 mg
Crystal Violet	2 mg
Agar	15 g

pH 7.4 ± 0.2

The agar medium is clear and reddish to violet coloured.

## Description

*Enterobacteriaceae* are producing acids from dextrose which is indicated by red coloured colonies and the formation of a precipitation zone around the colonies. Therefore red colonies are

usually *Enterobacteriaceae*. Only a few other bacteria of accompanying flora, e.g. *Aeromonas spp.* may show similar growth characteristics and should be differentiated.

Crystal violet and bile salts inhibit the accompanying bacterial flora widely.

An incubation time of more than 24 hours will decrease the selectivity of the medium.

## Culture Conditions

For test of absence test of bile tolerant gram-negative bacteria according to harmonised chapters of EP and USP the sample is diluted 1:10 in Tryptic Soy Broth (e.g. heipha ref. 502090-W). The dilution is incubated at 20-25°C for 2 to maximum 5 hours in order to resuscitate bacteria. Afterwards 10 ml of the dilution are transferred to Mossel Broth (e.g. heipha ref. 528100) and incubated at 30-35°C for 24-48 hours for selective enrichment. At least a subculture is made on VRBD Agar and incubated aerobically at 30-35°C for 18-24 hours.

For the quantitative test further 1:10 dilutions from the sample preparation described above are made in Tryptic Soy Broth. The diluted samples corresponding to 0.1 g or ml, 0.01 g or ml and 0.001 g or ml of the original sample are transferred to Mossel Broth and incubated for 24-48 hours at 30-35°C. Each selective enrichment culture is streaked out on VRBD Agar and incubated for 18-24 hours at 30-35°C. The most probable number of present bile-tolerant gram-negative bacteria is determined according to the smallest quantity of product which gives a positive result and the largest quantity of product which gives a negative result.

According to ISO 21528-1 for detection or enumeration of *Enterobacteriaceae* within in foodstuff using the MPN method the samples are suspended in Buffered Peptone Water (heipha ref.

193r or 507100). For MPN method further dilution steps are prepared. After pre-enrichment within Buffered Peptone Water a selective enrichment within Mossel Broth (ref. 2192r) is performed. At least all selective enrichment cultures are streaked on VRBD Agar and incubated for 22-26 hours at 37°C.

For colony count of *Enterobacteriaceae* within food stuffs according to ISO 21528-2 the sample is suspended in Buffered Peptone Water and further dilution steps are prepared in the same fluid. VRBD Agar in bottles (ref. 533200) is melted in a

water bath at 95°C for 45 minutes. Afterwards the agar is cooled down to 44-47°C in a second water bath for 45 minutes and maintained at this temperature for a maximum of 3.25 hours. The agar should be used within 4 hours maximum from the beginning of the melting process. 1 ml of each suspension is pipetted into a sterile empty petri dish and covered at latest after 15 minutes with 10 ml of the melted VRBD Agar and mixed carefully. After solidification further 15 ml of the fluid VRBD Agar are added. The plates are incubated for 22-26 hours at 37°C.

## Quality Control

Test Strains	Culture Conditions	Growth Characteristics
<i>Escherichia coli</i> ATCC 8739	21 ± 3h, 34 ± 1 °C 36 ± 1 °C (**)	good growth (recovery rate ≥ 50%), large red colonies with red precipitation zone
<i>Pseudomonas aeruginosa</i> ATCC 9027	21 ± 3h, 34 ± 1 °C 36 ± 1 °C (**)	good growth (recovery rate ≥ 50%), large colourless to pink colonies without precipitation zone
<i>Salmonella</i> Typhimurium ATCC 14028	21 ± 3h, 34 ± 1 °C 36 ± 1 °C (**)	good growth (recovery rate ≥ 50%), large red colonies with red precipitation zone
<i>Enterococcus faecalis</i> * ATCC 29212	21 ± 3h, 34 ± 1 °C 36 ± 1 °C (**)	no growth

Inoculum 10 - 100 CFU (Colony Forming Units), \* 10.000 - 100.000 CFU

\*\* Culture Conditions for reference numbers 185e, 245e and 533200

## Further Identification

According to EP and USP grown colonies give a positive result.

According to ISO 21528 *Enterobacteriaceae* will build characteristic pink or red colonies with or without precipitation zone. Some *Enterobacteriaceae* may grow to colourless or white colonies. The confirmation tests for suspect colonies are described within ISO 21528.

For testing oxidative and fermentative degradation of Glucose OF medium (Ref. 390r) can be used. The suspect colonies should be subcultured on Nutrient Agar free of carbohydrates (e.g. heipha ref. 309e or 3871e) before.

## References

European Pharmacopoeia 5.6 (Supplement 01/2007): 2.6.13. Test for specified micro-organisms.

ISO 21528-1 and -2 (2004-08): Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection and enumeration by MPN technique with pre-enrichment. - Part 2: Colony-count method.

Official Journal of the European Union L338/1-26 (2005): Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

United States Pharmacopoeia 30 NF25 (2007): <62> Microbiological examination of nonsterile products: Tests for specified microorganisms.