Technical Data Sheet

HEIMPLATE™ VRBD Agar

Ordering number: 1.46000.0020

90 mm settle plates is used for the detection and enumeration of Enterobacteriaceae from food and animal feed and other materials and bile-tolerant Gram-negative bacteria from pharmaceutical and other materials.

General

This culture medium complies with the specifications given by EN ISO 21528 and APHA. It complies with the specifications given by the harmonized methods of EP, USP, JP for Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms.
Violet Red Bile Dextrose (VRBD) agar is also named as Violet Red Bile Glucose (VRBG) agar or Crystal Violet Neutral Red Bile Glucose (Dextrose) agar.

Mode of Action

Crystal violet and bile salts inhibit the accompanying bacterial flora. Degradation of glucose is accompanied by production of acid, which is indicated by a color change to red and by zones of precipitated bile acids surrounding the colonies. All Enterobacteriaceae are detected as they all degrade glucose to acid. The culture medium is not, however, absolutely specific for these organisms as some other accompanying bacteria (e.g. Aeromonas) also show these reactions. Enzymatic digest of animal tissue provides carbon and nitrogen sources for the growth and yeast extract primarily supplies the B-complex vitamins.

Typical Composition (g/l)

Specified by ISO 21528 and APHA		**Specified by EP, USP, JP**		**Violet Red Bile Dextrose** HEIMPLATE™ VRBD agar	
Enzymatic Digest of Animal Tissues	7 g/l	Pancreatic Digest of Gelatin	7 g/l	Pancreatic Digest of Gelatin	7 g/l
Yeast Extract	3 g/l	Yeast Extract	3 g/l	Yeast Extract	3 g/l
Bile Salts No. 3	1.5 g/l	Bile Salts	1.5 g/l	Bile Salts	1.5 g/l
NaCl	5 g/l	NaCl	5 g/l	NaCl	5 g/l
Glucose	10 g/l	Glucose Monohydrate	10 g/l	Glucose Monohydrate	10 g/l
Neutral Red	0.03 g/l	Neutral Red	0.03 g/l	Neutral Red	30 mg/l
Crystal Violet	0.002 g/l	Crystal Violet	0.002 g/l	Crystal Violet	2 mg/l
Agar	9-18 g/l	Agar	15 g/l	Agar	15 g/l

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Water	1000 ml/l	Water	1000 ml/l	Water	1000 ml/l
pH at 25°C	7.4 ± 0.2	pH at 25°C	7.4 ± 0.2	pH at 25°C	7.4 ± 0.2

Application and Interpretation

Please check each agar plate on sterility before using it and pay attention to aseptic handling in order to avoid false positive results.

Pharma

For test of absence of bile tolerant gram-negative bacteria according to harmonized chapters of EP and USP, the sample is diluted 1:10 in Tryptic Soy Broth.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 and incubated at 30-35°C for 24-48 hours for selective enrichment. At last, 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 EP and USP grown colonies give a positive result

ISO

According to ISO 21528-1 for detection or enumeration of Enterobacteriaceae within foodstuff using the MPN method, the samples are diluted and enriched in Buffered Peptone Water. After incubation at 37 (or 30) °C for 16-20 h, each enrichment culture is streaked for isolation onto

VRBD Agar and incubated aerobically for 22-26 hours at 37 (or 30) °C.

Enterobacteriaceae create characteristic pink or red colonies with or without precipitation zone. Some Enterobacteriaceae may grow to colorless or white colonies.

Suspect colonies (or a representative proportion thereof) may subjected to confirmatory tests. The confirmation tests for suspect colonies are described within ISO 21528.

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +15°C to +25°C.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

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The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121°C, disinfect, incinerate etc.).

Quality Control for Pharma

Control Strains	ATCC #	InoculumCFU	Incubation	Expected Results
Escherichia coli	8739	10-100	16-18 h at30-35°C	50-200%
Salmonella Typhimurium	14028	10-100	16-18 h at30-35°C	50-200%
Escherichia coli	8739	10-100	18-24 h at30-35°C	good growth; reddish colonies withprecipitation zone
Salmonella Typhimurium	14028	10-100	18-24 h at30-35°C	good growth; reddish colonies withprecipitation zone

Quality Control for ISO 11133 Compliance

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Escherichia coli* ATCC 8739 (WDCM 00012)	22-26 h at 36-38°C	Tryptic Soy Agar (TSA) / Columbia Blood Agar	Quantitative	Recovery ≥50%, pink to redcolonies with or without precipitation halo
	Escherichia coli ATCC 25922 (WDCM 00013)				
	Salmonella Typhimurium ATCC 14028 (WDCM 00031)				
	Salmonella Enteritidis ATCC 13076 (WDCM 00030)				
	Enterococcus faecalis				

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Selectivity	ATCC 19433 (WDCM 00009) *Enterococcus faecalis* ATCC 29212 (WDCM 00087)	22-26 h at 36-38°C	Tryptic Soy Agar (TSA) / Columbia Blood Agar	Qualitative	Total inhibition

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133. It is advisable to use freshly prepared cultures for testing the medium in order to receive best results.

A recovery rate of 50% is equivalent to a productivity value of 0.5.

Salmonella Typhimurium ATCC 14028

Escherichia coli ATCC 8739

Literature

APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

EU GMP Medicinal Products for Human and Veterinary use (2008): Annex1 Manufacture of Sterile Medicinal Products.

European Directorate for the Quality of Medicines and Healthcare. (2019): The European Pharmacopoeia. 10th Ed. Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice.

ISO International Standardisation Organisation. 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. EN ISO 21528-1:2017.

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Japanese Ministry of Health, Labour and Welfare. (2016): The Japanese Pharmacopoeia. 17th Ed. Chapter 4.05 Microbial Limit Test II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

United States Pharmacopoeia 42 NF 37 (2019): <62> Microbiological examination of non-sterile products: Tests for specified microorganisms.

Ordering Information

Product	Cat. No.	Pack size
HEIMPLATE™ VRBD Agar	1.46000.0020	20 x 90 mm
GranuCult™ VRBD Agar EP,USP, JP, ISO 21528	1.10275.0500	500 g

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