VIOLET RED BILE AGAR (VRBA) WITH GLUCOSE

PRODUCT:

Plated Media:

Violet Red Bile Agar with Glucose P8945

PURPOSE:

Violet Red Bile Agar (VRBA) with Glucose is used for the detection and enumeration of *Enterobacteriaceae* in food and dairy products. This media meets the U.S. Pharmacopeia (USP) standards for use as a selective media in performing microbial examination of nonsterile products.

PRINCIPLE:

Enterobacteriaceae are lactose-fermenting bacteria. However, Mossel et al.[1,2] demonstrated that substituting lactose for glucose can improve the recovery of *Enterobacteriaceae* in food and dairy products. The selectivity of the media is due to the presence of bile salts and crystal violet. Degradation of glucose to acid is indicated by the pH indicator neutral red, which changes its color to red-purple, and by precipitation of bile acids, indicated by a halo of the same color. Non-glucose fermenters produce pale to colorless colonies. Selectivity can be increased by incubation at 42-44°C.

FORMULA:

Approximate, per liter deionized filtered water.

Violet Red Bile Agar with Glucose:

Yeast Extract	3.0 g
Pancreatic Digest of Gelatin	7.0
Bile Salts	1.5
Sodium Chloride	5.0
Glucose Monohydrate	10.0
Agar	15.0
Crystal Violet	2.0 mg
Neutral Red	30.0

Final pH 7.4 ± 0.2 at 25°C

PRECAUTIONS:*

For *in vitro* diagnostic use only. Not intended for use in the diagnosis of disease or other conditions. Observe approved biohazard precautions.

Storage: Upon receipt, store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: Using VRBA with Glucose is only part of the identification. Biochemical and serological testing are necessary for definitive identification of microorganisms.

A few other bacteria of accompanying flora, such as *Aeromonas spp.*, may exhibit similar growth characteristics as *Enterobacteriaecae* and should be further differentiated.

An incubation time of more than 24 hours may decrease the selectivity of the media.

Injured cells may not grow well on VRBA with Glucose when directly inoculated. The use of an enrichment media to resuscitate cells is recommended before subculturing onto VRBA with Glucose.

PROCEDURE:*

Specimen Collection: Information on specimen collection and preparation can be found in standard reference materials, such as FDA guidelines and in USP texts. In general, specimens should be protected from extreme heat and cold and should be delivered to the laboratory without delay.

A selective broth media, such as Enterobacteria Enrichment Broth Mossel, can be used for sample preparation and enrichment.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Inoculate the media in such a manner as to facilitate isolation of individual colonies. Four-quadrant streaking is recommended for maximum isolation. Incubate aerobically at 32.5°C +/- 0.5°C and examine plates at 18-24 hours.

Interpretation:
Enterobacteriaceae ferment lactose/glucose and produce acid products. Typically, red-purple colonies form surrounded by a red-purple halo.

Microorganisms	Appearance of Colonies
Lactose-positive *Enterobacteriaceae*: coliform bacteria, *E. coli*	Red-purple, surrounded by reddish precipitation zones, diameter 1-2 mm
Enterococci, possibly *Klebsiella*	Pink pin-point colonies
Lactose-negative *Enterobacteriaceae*	Colorless

Materials Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, and incinerator, are not provided. Enrichment media, such as Enterobacteria Enrichment Broth Mossel, is not provided.

QUALITY CONTROL:*

Violet Red Bile Agar with Glucose

Microorganisms Used (ATCC #):	**Expected Results:**
Escherichia coli (8739)	Growth; pink colonies with bile precipitate
Pseudomonas aeruginosa (9027)	Growth; colorless to pink colonies with no bile precipitate

User Quality Control: Check for signs of contamination and deterioration. Violet Red Bile Agar with Glucose should appear hazy to slightly opalescent, and red to purple in color.

BIBLIOGRAPHY:

1. Mossell, et al. 1978. Lab Practice. *27*:1049.
2. Mossell, et al. 1979. J. Food Protection. *42*:470.
3. United States Pharmacopeia 30 - NF 25, Chapter 62, Microbial examination of nonsterile products: Tests for specified microorganisms, 2007.
4. Wehr, H.M., and J.F. Frank, (eds.), Standard Methods for the Examination of Dairy Products, 17th ed., American Public Health Association, Washington, D.C., 2004.

*For more detailed information, consult appropriate references.

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