

Violet Red Bile Agar

Intended Use

Violet Red Bile Agar is used for enumerating coliform organisms in dairy products.

Summary and Explanation

The coliform group of bacteria includes aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Members of the *Enterobacteriaceae* comprise the majority of the group but other lactose fermenting organisms may also be included.

Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.¹⁻³ One method for performing the presumptive test for coliforms uses

Violet Red Bile Agar. If typical coliform colonies appear, they are tested further to confirm their identification as coliforms.

Principles of the Procedure

Violet Red Bile Agar contains peptone to provide carbon and nitrogen sources for general growth requirements. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Bile salts and crystal violet inhibit most gram-positive microorganisms. Lactose is the carbohydrate source and neutral red is the pH indicator. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ Violet Red Bile Agar

Dehydrated Appearance: Beige to reddish-beige, homogeneous, free-flowing.

Solution: 4.15% solution, soluble in purified water upon boiling. Solution is reddish-purple, slightly opalescent, without significant precipitate.

Prepared Appearance: Reddish-purple, slightly opalescent, no significant precipitate.

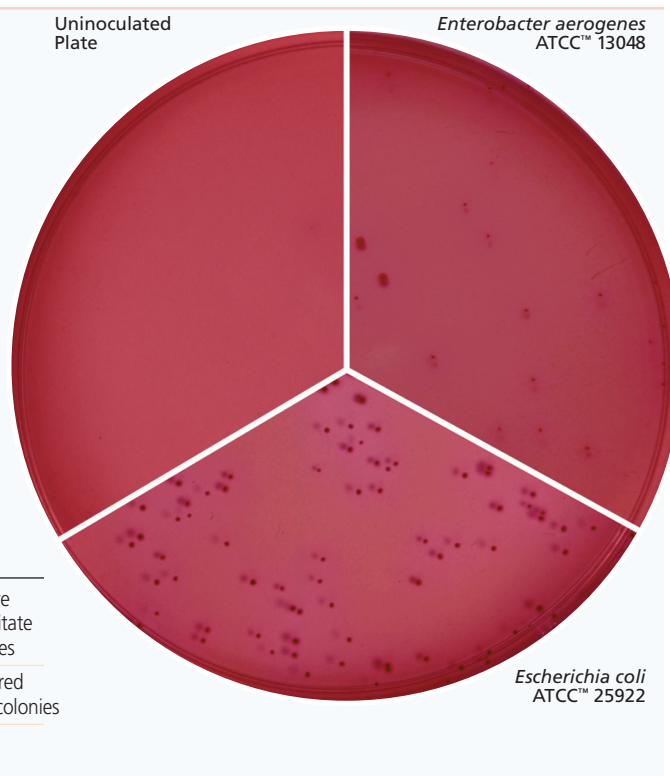
Reaction of 4.15% Solution at 25°C: pH 7.4 ± 0.2

Cultural Response

Difco™ Violet Red Bile Agar

Prepare the medium per label directions. Inoculate and incubate at 32 ± 1°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterobacter aerogenes</i>	13048	30-300	Good	Red, may have slight red precipitate around colonies
<i>Escherichia coli</i>	25922	30-300	Good	Deep red with red precipitate around colonies
<i>Staphylococcus aureus</i>	25923	~10 ³	Marked to complete inhibition	–



Formula

Difco™ Violet Red Bile Agar

Approximate Formula* Per Liter	
Yeast Extract	3.0 g
Peptone	7.0 g
Bile Salts No. 3	1.5 g
Lactose	10.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Neutral Red	0.03 g
Crystal Violet	2.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 41.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Presumptive test for coliforms using solid medium:

1. Transfer a 1 mL aliquot of test sample to a Petri dish.
2. Add 10 mL of Violet Red Bile Agar (at 48°C) and swirl to mix.
3. Allow medium to solidify before incubating at 35°C for 18-24 hours; use 32°C for dairy products.
4. Examine for purple-red colonies, 0.5 mm in diameter (or larger), surrounded by a zone of precipitated bile acids.
5. Continue with confirmatory testing of typical coliform colonies.¹⁻³

Expected Results

Lactose fermenters: Purple-red colonies, with or without a zone of precipitate around the colonies

Lactose nonfermenters: Colorless to transparent colonies

Gram-positive cocci: Colorless, pinpoint colonies

Limitations of the Procedure

1. Violet Red Bile Agar may not be completely inhibitory to gram-positive organisms. Perform Gram stain and biochemical tests as necessary to identify isolates.
2. The medium will grow gram-negative bacilli other than members of the *Enterobacteriaceae*. Perform biochemical tests to identify isolates to genus and species.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.
4. Plates of Violet Red Bile Agar should not be incubated longer than 24 hours because microorganisms that are only partially inhibited may grow after extended incubation.
5. For optimum performance, prepare and use the medium within 24 hours.

References

1. Davidson, Roth, and Gambrel-Lenarz. 2004. *In* Wehr and Frank (ed.). Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Kormacki and Johnson. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Violet Red Bile Agar

BAM **CCAM** **COMPF** **ISO** **SMD**

Cat. No. 211695 Dehydrated – 500 g
211687 Dehydrated – 2 kg

Mexico

Cat. No. 252633 Prepared Bottles, 140 mL – Pkg. of 12