# Violet Red Bile Agar
## SKU: 700003032, 700003033, 700003034, 700003035
## (NCM0025)

**Intended Use**

Violet Red Bile Agar is used for the enumeration of coliforms in food and dairy products in a laboratory setting. Violet Red Bile Agar is not intended for use in the diagnosis of disease or other conditions in humans and conforms to American Public Health Association (APHA).

**Description**

The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli. Coliforms ferment lactose and form acid and gas at 35°C within 48 hours. Members of *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. One method for performing the presumptive test for coliforms uses Violet Red Bile Agar, (VRBA). If typical coliform colonies appear, they are tested further to confirm their identification as coliforms. Coliform colonies lower the pH of the medium, subsequently causing their colonies to look red (Neutral Red Dye) and to precipitate the bile salts.

**Typical Formulation**

| | |
|---|---|
| Yeast Extract | 3.0 g/L |
| Enzymatic Digest of Gelatin | 7.0 g/L |
| Bile Salts | 1.5 g/L |
| Lactose | 10.0 g/L |
| Sodium Chloride | 5.0 g/L |
| Neutral Red | 0.03 g/L |
| Crystal Violet | 0.002 g/L |
| Agar | 15.0 g/L |

Final pH: 7.4 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

**Precaution**

Refer to SDS

**Preparation**

1. Suspend 41.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for two minutes. DO NOT AUTOCLAVE.
3. Cool the medium to 45 - 50°C and dispense 15 - 20 mL into 100 mm petri dishes containing inoculum.
4. After solidification of the inoculated medium, evenly add a cover layer of 4 mL of the cooled (45 -50°C) agar medium.

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## Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and beige to reddish-beige.

**Prepared Appearance:** Prepared medium is clear to slightly hazy and red-purple.

**Expected Cultural Response:** Cultural response on Violet Red Bile Agar following incubation aerobically at 33-38°C and examined for growth after 18 - 24 hours.

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|---|---|---|---|
| | | Growth | Reaction |
| *Enterobacter aerogenes* ATCC® 13048 | 10 - 300 | Fair to Good | Pink colonies |
| *Enterococcus faecalis* ATCC® 29212 | $10^3$ | Complete Inhibition | ---- |
| *Escherichia coli* ATCC® 25922 | 10 - 300 | Good to Excellent | Pink to red colonies w/ bile precipitate |
| *Salmonella typhimurium* ATCC® 14028 | 10 - 300 | Fair to Good | Colorless colonies |

The organisms listed are the minimum that should be used for quality control testing.

**Expected Cultural Response:** Tested per ISO 11133:2014. Cultural response on Violet Red Bile Agar following incubation aerobically at 30 ± 1°C and examined for growth after 22 - 26 hours.

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|---|---|---|---|
| | | Recovery | Reaction |
| *Enterococcus faecalis* ATCC® 29212 | $10^4$-$10^6$ | Complete Inhibition | --- |
| *Enterococcus faecalis* ATCC® 19433 | $10^4$-$10^6$ | Complete Inhibition | --- |
| *Escherichia coli* ATCC® 25922 | < 100 | Recovery > 50% | Pink to red colonies w/ bile precipitate |
| *Escherichia coli* ATCC® 8739 | < 100 | Recovery > 50% | Pink to red colonies w/ bile precipitate |
| *Pseudomonas aeruginosa* ATCC® 27853 | $10^3$-$10^4$ | Growth | Colorless colonies |

## Test 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 45-50°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 precipitate bile acids. Continue with confirmatory testing of typical organism colonies.

**Results**

Lactose fermenters are purple-red, with or without a zone of precipitate around the colonies. Lactose non-fermenters are colorless to transparent colonies. Gram-positive cocci are colorless, pinpoint colonies.

**Expiration**

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitations of the Procedure**

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. VRBA may not be completely inhibitory to Gram-positive organisms and will grow Gram-negative bacilli other than members of *Enterobacteriaceae*. Perform a Gram stain and biochemical tests to identify isolates.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.

**Storage**

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

**References**

1. Marshall, R. T. (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.