Rose Bengal Agar Base
Rose Bengal Antimicrobic Supplement C

Intended Use

Rose Bengal Agar Base is used with Rose Bengal Antimicrobic Supplement C in isolating and enumerating yeasts and molds.

Summary and Explanation

A number of methods have been described for the selective isolation of fungi from environmental materials and foodstuffs containing mixed populations of fungi and bacteria. The use of media with an acid pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed.[1-3] A number of investigators have reported, however, that acidified media may actually inhibit fungal growth,[4,5] fail to completely inhibit bacterial growth[5] and have little effect in restricting the size of mold colonies.[6] Smith and Dawson[7] used rose bengal in a neutral pH medium for the selective isolation of fungi from soil samples. Chloramphenicol, streptomycin, oxytetracycline and chlortetracycline have been used for the improved, selective isolation and enumeration of yeasts and molds from soil, sewage and foodstuffs.[4,8-11]

Rose Bengal Agar Base supplemented with Rose Bengal Antimicrobic Supplement C is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis.[11] Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Of the antibiotics most frequently employed in media of neutral pH, chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum. A modified formulation of Rose Bengal Agar is recommended in *Standard Methods for the Examination of Water and Wastewater* for the enumeration of yeasts and molds.[12]

User Quality Control

Identity Specifications

Difco™ Rose Bengal Agar Base

Dehydrated Appearance:	Beige to faint pink, free-flowing, homogeneous.
Solution:	3.2% solution, soluble in purified water upon boiling. Solution is reddish pink, very slightly to slightly opalescent.
Prepared Appearance:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.2% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Rose Bengal Antimicrobic Supplement C

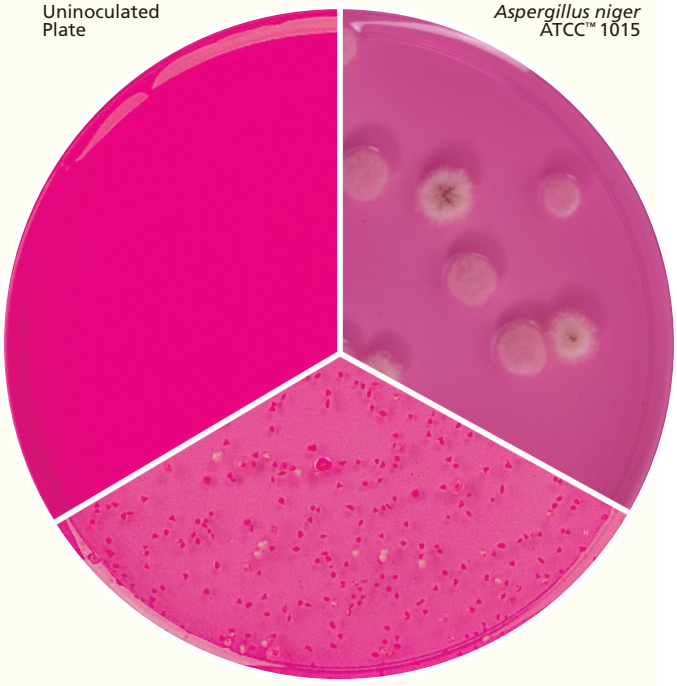
Lyophilized Appearance:	White cake, may be dispersed.
Rehydrated Appearance:	Colorless, clear.
Solubility:	Soluble in 3 mL ethanol.

Cultural Response

Difco™ Rose Bengal Agar Base with Antimicrobic Supplement C

Prepare the medium per label directions. Inoculate using the pour plate technique (for *Aspergillus niger*, inoculate the surface of an agar slant) and incubate aerobically at 25-30°C for up to 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Aspergillus niger	1015	Fresh	Good	White to black
Candida albicans	10231	10^2-3×10^2	Good	Pink
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition	–
Micrococcus luteus	10240	10^3-2×10^3	Marked to complete inhibition	–



The Hycheck™ hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are two slides with Rose Bengal Chloramphenicol Agar along with another medium: Tryptic Soy Agar; and Tryptic Soy Agar with 0.01% TTC.

Principles of the Procedure

Peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source. Monopotassium phosphate provides buffering capability. Magnesium sulfate provides necessary trace elements. Rose bengal is included as a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing molds. The restriction in growth of molds aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. Rose bengal is taken up by yeast and mold colonies, thereby facilitating their recognition and enumeration. Rose Bengal Antimicrobic Supplement C is a lyophilized antimicrobic supplement containing chloramphenicol which inhibits bacteria. Agar is the solidifying agent.

Formulae

Difco™ Rose Bengal Agar Base

Approximate Formula* Per Liter		
Soy Peptone	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Rose Bengal	0.05	g
Agar	15.0	g

Difco™ Rose Bengal Antimicrobic Supplement C

Approximate Formula* Per 3 mL Vial		
Chloramphenicol	0.05	g

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

Directions for Preparation from Dehydrated Product

Difco™ Rose Bengal Agar Base

1. Suspend 16 g of the powder in 500 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 3 mL rehydrated Rose Bengal Antimicrobic Supplement C to 500 mL of cooled agar base (final concentration of chloramphenicol is 100 mg/L). Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Rose Bengal Antimicrobic Supplement C (Chloramphenicol)

1. Aseptically add 3 mL ethanol per vial of supplement.
2. Invert several times to dissolve the powder.

Procedure

1. Inoculate 0.1 mL of appropriate dilutions in duplicate on the solidified agar. Spread over the entire surface using a sterile bent glass rod.
2. Incubate plates at 25-30°C for up to 7 days.

Expected Results

Colonies of yeast appear pink due to the uptake of rose bengal. Count plates containing 15-150 colonies and report the counts as colony-forming units (CFU) per gram or mL of sample.

Limitations of the Procedure

1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light, since photodegradation of rose bengal yields compounds that are toxic to fungi.[13]

References

1. Waksman. 1922. J. Bacteriol. *7*:339.
2. Koburger. 1976. *In* Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
3. Mossel, Visser and Mengerink. 1962. Lab Practice *11*:109.
4. Martin. 1950. Soil Sci. *69*:215.
5. Koburger. 1972. J. Milk Food Technol. *35*:659.
6. Tyner. 1944. Soil Sci. *57*:271.
7. Smith and Dawson. 1944. Soil Sci. *58*:467.
8. Cooke. 1954. Antibiot. and Chemother. *4*:657.
9. Papavizas and Davey. 1959. Soil Sci. *88*:112.
10. Overcast and Weakley. 1969. J. Milk Technol. *32*:442.
11. Jarvis. 1973. J. Appl. Bacteriol. *36*:723.
12. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
13. Banks, Board and Paton. 1985. Lett. Appl. Microbiol. *1*:7.

Availability

Difco™ Rose Bengal Agar Base

SMWW

Cat. No. 218312 Dehydrated – 500 g

Difco™ Rose Bengal Antimicrobic Supplement C

SMWW

Cat. No. 214904 Vial – 10 × 3 mL*

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No. 290006 Rose Bengal Chloramphenicol Agar// Tryptic Soy Agar – Pkg. of 10 slides*

290007 Rose Bengal Chloramphenicol Agar// Tryptic Soy Agar with 0.01% TTC – Pkg. of 10 slides*

**Store at 2-8°C.*