Cooke Rose Bengal Agar Antimicrobic Vial A

Intended Use

Cooke Rose Bengal Agar is used with or without Antimicrobic Vial A in isolating fungi from environmental and food specimens.

Antimicrobic Vial A is used in preparing microbiological culture media.

Summary and Explanation

Cooke Rose Bengal Agar is a selective medium for the isolation of fungi prepared according to the formula of Cooke.[1,2] Selectivity of the medium is increased by the addition of antibiotics.

A variety of materials and methods have been used to inhibit bacteria in an attempt to isolate fungi from mixed flora. Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[3] Waksman[4] described an acid medium consisting of peptone, dextrose, inorganic salts and agar for the isolation of fungi from soil. Cooke[1] used the Waksman[4] medium without adjustment to investigate the isolation of fungi from sewage. It was discovered that soy peptone was particularly suitable for use in this medium and that the combination of chlortetracycline, or oxytetracycline, with rose bengal increased the selectivity of the medium.

Antimicrobic Vial A contains sterile, desiccated chlortetracycline. It was originally used in preparing DTM Agar described by Taplin, Azias, Rebell and Blank[5] for the isolation of dermatophytes. Antimicrobic Vial A is applicable for use in various media requiring this antibiotic. Cooke[1] preferred chlortetracycline in Cooke Rose Bengal Agar due to the increased stability of the antibiotic.

Principles of the Procedure

Peptone provides nitrogen, carbon and vitamins in Cooke Rose Bengal Agar. Dextrose is an energy source. Rose bengal and chlortetracycline selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing molds. Monopotassium phosphate provides buffering capability. Magnesium sulfate is a source of divalent cations. Agar is the solidifying agent.

Formulae

Difco™ Cooke Rose Bengal Agar

Approximate Formula* Per Liter

Soy Peptone	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Agar	20.0	g
Rose Bengal	35.0	mg

Difco™ Antimicrobic Vial A

Contains 25 mg sterile desiccated chlortetracycline per 10 mL vial.

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

User Quality Control

Identity Specifications

Difco™ Cooke Rose Bengal Agar

Dehydrated Appearance:	Pinkish-tan, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is pinkish red, slightly opalescent.
Prepared Appearance:	Deep pink, slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.0 ± 0.2

Difco™ Antimicrobic Vial A

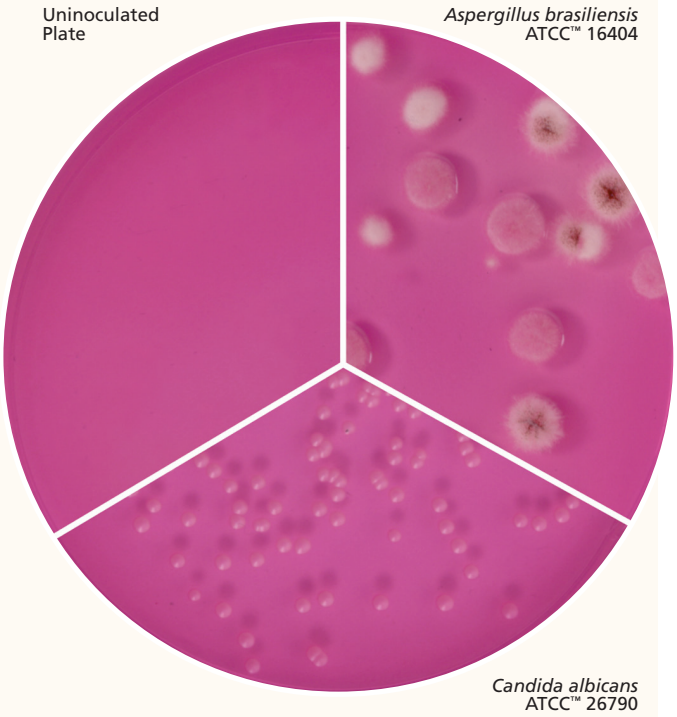
Desiccated Appearance:	Yellow cake or powder.
Rehydrated Appearance:	Yellow, clear solution.
Solution:	Soluble in 10 mL purified water.

Cultural Response

Difco™ Cooke Rose Bengal Agar and Antimicrobic Vial A

Prepare the medium with 35 µg per mL chlortetracycline (Antimicrobic Vial A added aseptically) per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	Undiluted	Good
Candida albicans	26790	30-300	Good
Escherichia coli	25922	10^3	Inhibition
Saccharomyces cerevisae	9763	30-300	Good



Directions for Preparation from Dehydrated Product

Difco™ Cooke Rose Bengal Agar

1. Suspend 36 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.
3. Autoclave at 121°C for 15 minutes. Cool to 45°C.
4. Aseptically add 14 mL of rehydrated Antimicrobic Vial A (final concentration of 35 µg chlortetracycline per mL) or appropriate amount of another antibiotic.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Antimicrobic Vial A

1. Aseptically add 10 mL sterile purified water per vial of supplement.
2. Agitate gently to dissolve completely.
3. The resulting concentration of the rehydrated solution is 2.5 mg chlortetracycline per mL.

Procedure

Refer to appropriate references for specific procedures on the isolation and cultivation of fungi.

Expected Results

Refer to appropriate references and procedures for results.

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 photo-degradation of rose bengal yields compounds that are toxic to fungi.[6,7]

References

1. Cooke. 1954. Antibiot. Chemother. *4*:657.
2. 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.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Waksman. 1922. J. Bacteriol. *7*:339.
5. Taplin, Azias, Rebell and Blank. 1969. Arch. Dermatol. *99*:203.
6. Banks, Board and Paton. 1985. Lett. Appl. Microbiol. *1*:7.
7. Beuchat and Cousin. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Cooke Rose Bengal Agar

SMWW

Cat. No. 270310 Dehydrated – 500 g

Difco™ Antimicrobic Vial A

SMWW

Cat. No. 233331 Vial – 6 x 10 mL*

**Store at 2-8°C.*