DRBC Agar

Intended Use
DRBC Agar is used for the enumeration of yeasts and molds.

Summary and Explanation
DRBC (Dichloran Rose Bengal Chloramphenicol) Agar is based on the Dichloran Rose Bengal Chlortetracycline Agar formula described by King, Hocking and Pitt.[1] DRBC Agar conforms with APHA guidelines for the mycological examination of foods, containing chloramphenicol rather than chlortetracycline as originally proposed.[2] DRBC Agar is a selective medium that supports good growth of yeasts and molds.

Principles of the Procedure
Peptone provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate is a buffering agent. Magnesium sulfate is a source of divalent cations and sulfate. The antifungal agent, dichloran, is added to the medium to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of the spreading fungi.[1] The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing molds. The concentration of rose bengal is reduced from 50 µg/mL to 25 µg/mL as found in Rose Bengal Chloramphenicol Agar for optimal performance with dichloran. Chloramphenicol is included in this medium to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing molds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. In addition, rose bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated. Reduced recovery of yeasts may be encountered due to increased activity of rose bengal at pH 5.6.[1] Agar is the solidifying agent.

Formula
Difco™ DRBC Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Dichloran	2.0	mg
Rose Bengal	25.0	mg
Chloramphenicol	0.1	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product
1. Suspend 31.6 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.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[2,3]
1. Inoculate 0.1 mL of appropriate decimal dilutions of the sample in duplicate onto the surface of DRBC Agar plates. The plates should be dried overnight at room temperature. Spread the inoculum over the entire surface of the plate using a sterile, bent-glass rod.
2. Incubate plates upright at 22-25°C. Examine for growth of yeasts and molds after 3, 4 and 5 days incubation.

Expected Results
Colonies of molds and yeasts should be apparent within 5 days of incubation. Colonies of yeast appear pink due to the uptake of rose bengal. Report the results as colony-forming units per gram or milliliter of sample.

User Quality Control

Identity Specifications
Difco™ DRBC Agar

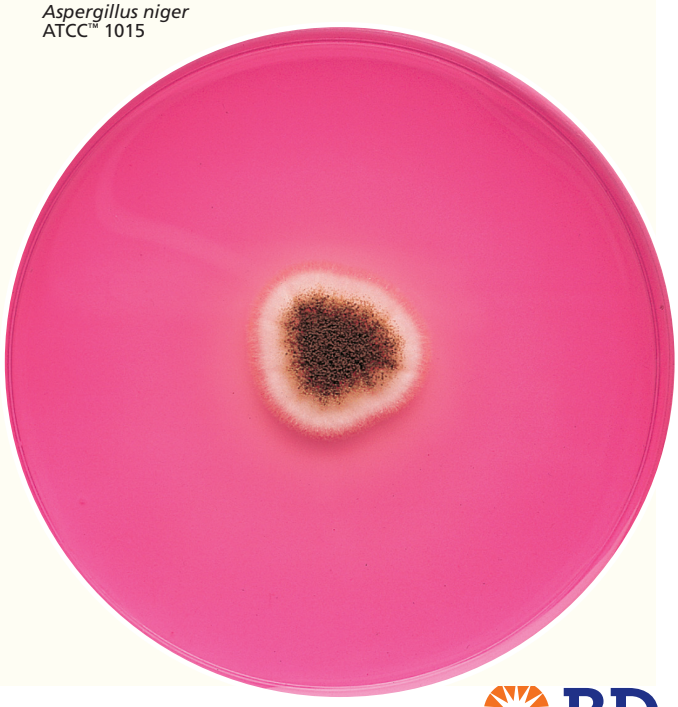
Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	3.16% solution, soluble in purified water upon boiling.Solution is reddish pink, very slightly to slightly opalescent.
Prepared Appearance:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.16% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response
Difco™ DRBC Agar

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for up to 5 days. For *A. niger*, spot inoculate.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	1015	Undiluted	Good
Candida albicans	10231	10^2-10^3	Good
Escherichia coli	25922	10^3	None to poor
Micrococcus luteus	10240	10^3	None to poor

Aspergillus niger
ATCC™ 1015



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 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.[2-4]

References

1. King, Hocking and Pitt. 1979. Appl. Environ. Microbiol. *37*:959.
2. 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.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Banks, Board and Paton. 1985. Lett. Appl. Microbiol. *1*:7.

Availability

Difco™ DRBC Agar

BAM CCAM COMPF SMD

Cat. No. 258710 Dehydrated – 500 g