Rose Bengal Chloramphenicol HiVeg® Agar

MV640

Intended use

Recommended for selective isolation and enumeration of yeasts & moulds from food, environmental materials and other samples.

Composition**

Ingredients	g / L
HiVeg® peptone No. 4	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Rose bengal	0.050
Chloramphenicol	0.100
Agar	15.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.15 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Rose Bengal Chloramphenicol HiVeg® Agar is prepared by using HiVeg® peptone No. 4 in place of mycological peptone which makes the medium free of BSE/TSE risks. It is the modification of Rose Bengal Chloramphenicol Agar which was formulated originally by Jarvis (1) and further modified by Overcast and Weakley (2). The use of rose bengal in the media having neutral pH was reported by Smith and Dawson (3). HiVeg® peptone No.4 provides carbon, nitrogen substances, long chain amino acids, vitamins and other essential growth nutrients. Dextrose is the energy source. Chloramphenicol has inhibitory action on gram-negative bacteria. Rose bengal dye suppresses the development of bacteria and reduces the spreading of moulds, controlling size and height of moulds colonies such as *Rhizopus* species (4). The medium has neutral pH which with antibiotics have noted to be advantageous (5, 6). Rose bengal is taken up by moulds and yeast colonies thereby assisting in enumeration (1). The number of yeasts or moulds is calculated per 1 gram or 1 ml of sample to be tested by multiplying the number of colonies by dilution factor. Colonies of bacteria and yeasts could be confused by appearance and thus should be examined microscopically. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may grow poorly or fail to grow on the complete medium; similarly, some strains of bacteria might not be inhibited or only partially inhibited. 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).

Type of specimen

Food samples; Environmental samples.

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. The medium should not be exposed to light, since photo degradation of rose bengal yields compounds that are toxic to fungi.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.55% Agar gel.

Colour and Clarity of prepared medium

Deep pink coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.22% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 5 days.

Organism	Inoculum (CFU)	Growth
# *Aspergillus brasiliensis* ATCC 16404 (00053*)	50-100	good-luxuriant
** *Bacillus spizizenii* ATCC 6633 (00003*)	>=10^4	inhibited
*Cladosporium cladosporioides*ATCC 11278	50-100	good-luxuriant
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited
Mucor racemosus ATCC 42647	50-100	good-luxuriant
Pencillium notatum ATCC 10108	50-100	good-luxuriant
Saccharomyces cerevisiae ATCC 9763	50-100	good-luxuriant

Key: (#)Formerly known as *Aspergillus niger* (**)Formerly known as *Bacillus subtilis* subsp. *spizizenii*

(*) Corresponding WDCM numbers

Storage and Shelf Life

Store the dehydrated and prepared media between 15-25°C in a tightly closed container . Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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