

## Rose Bengal Chloramphenicol Agar Plate

MP640

### Intended use

For selective isolation and enumeration of yeasts and moulds from foods and environmental materials.

### Composition\*\*

Ingredients	Gms / Litre
Mycological peptone	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

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

### Principle And Interpretation

Rose Bengal Chloramphenicol Agar 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). Mycological peptone provides carbon, nitrogen substances, long chain amino acids, vitamins and other essential growth nutrients. Dextrose (Glucose) is the fermentable carbohydrate. Chloramphenicol has inhibitory action on gram-negative bacteria. Rose bengal dye suppresses the development of bacteria and reduces the spreading of moulds, controls the size and height of moulds colonies such as *Rhizopus* species (2). The medium has neutral pH, which with the antibiotics has noted to be advantageous (4,5). Rose bengal is taken up by moulds and yeast colonies thereby assist in enumeration (6).

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 may also not 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 (7,3).

### 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 (6).

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 photodegradation of rose bengal yields compounds that are toxic to fungi.
- 2.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
- 3.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
- 4.Further serological and biochemical testing is required for complete identification.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within expiry period when stored at the recommended temperature.

## Quality Control

### Appearance

Sterile Rose Bengal Chloramphenicol Agar in 90 mm disposable plates with smooth surface and absence of black particles/ cracks/bubbles

### Colour of medium

Deep pink coloured medium.

### Quantity of medium

25 ml of medium in 90 mm disposable plates.

### pH

7.00-7.40

### Sterility Check

Passes release criteria

### Cultural Response

Cultural characteristics observed after an incubation at 25-30°C (22-28 in coa) for 5 days. (48-72hrs in coa)

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

#Key: Formerly known as *Aspergillus niger*, \* Corresponding WDCM numbers

## Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. 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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