



Rose Bengal Agar Base

M842

Intended Use:

Recommended for the selective isolation and enumeration of yeasts and moulds from environmental materials, foodstuff and clinical samples.

Composition**

Ingredients	g / L
Soya peptone	5.000
Dextrose (Glucose)	10.000
Monopotassium phosphate	1.000
Magnesium sulphate	0.500
Rose bengal	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.55 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. Aseptically add the rehydrated contents of one vial of Chlor Selective Supplement (FD033) for each 500 ml of medium. Mix thoroughly and pour into sterile Petri plates.

Principle And Interpretation

Rose Bengal Agar is a selective medium to detect and enumerate yeasts and moulds in food samples. The use of media with an acidic pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed (1-3). Neutral pH media with antibiotics is advantageous for fungal growth compared to acidified media as the later may inhibit fungal growth or fail to inhibit bacterial growth (4,5) and may restrict the size of mould 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 moulds from soil, sewage and foodstuffs (5,8,9,10). Rose Bengal Agar Base supplemented with chloramphenicol is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis (9). Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum (11). Rose Bengal Agar is recommended in standard methods for the enumeration of yeasts and moulds from foodstuffs and water (2,).

Soya 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 sulphate provides necessary trace elements. Rose bengal is a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing moulds. Rose bengal is taken up by yeast and mould colonies, thereby facilitating their recognition and enumeration. Chlor Selective Supplement (FD033) inhibit bacteria. 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 photodegradation of rose bengal yields compounds that are toxic to fungi (2,13).

Type of specimen

Food samples; Environmental samples, Clinical samples -skin scrapings

Specimen Collection and Handling:

Add 1 ml aliquots of a suitable series of dilution to Petri plates. Pour the cooled medium, mix well and incubate for upto 5 days at 25°C. Calculate the number of yeasts or moulds per 1 gram or 1 ml by multiplying the number of colonies by dilution factor. Colonies of yeast appear pink due to uptake of rose bengal. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. 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.
3. The medium should not be exposed to light, since photodegradation of rose bengal yields compounds that are toxic to fungi. (1,12)

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.16% 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 20-25°C for 5 days with added Chlor Selective Supplement (FD033).

Organism	Inoculum(CFU)	Growth	Recovery
# <i>Aspergillus brasiliensis</i> ATCC 16404 (00053*)	50-100	good	
<i>Candida albicans</i> ATCC 10231 (00054*)	50-100	good	≥50%
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%
<i>Micrococcus luteus</i> ATCC 10240	≥10 ⁴	inhibited	0%
<i>Saccharomyces cerevisiae</i> ATCC 9763 (00058*)	50-100	good	≥50%

Key: *Corresponding WDCM numbers

Formerly known as *Aspergillus niger*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (13,14).

Reference

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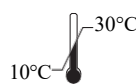
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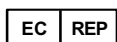
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