

HiDip™ PCA W/ TTC & Neutralizers-Rose Bengal

HD029

Chloramphenicol Agar w/ Neutralizers

For total bacterial count, yeast and moulds with inactivation of disinfectants.

Composition**

Ingredients	Gms / Litre
PCA w/ TTC & Neutralizers	-
Casein enzymic hydrolysate	5.000
Yeast extract	2.500
Dextrose	10.000
Agar	15.500
Sodium Thiosulphate	0.004
Tween 80	0.150
TTC 4%	1.300
Rose Bengal Chloramphenicol Agar w/Neutralizers	-
Mycological peptone	5.000
Monopotassium phosphate	1.000
Magnesium sulphate heptahydrate	0.500
Rose bengal	0.050
Chloramphenicol	0.100
Sodium Phosphate Bibasic	0.200
Lecithin	0.015
L- Histidine	0.005

**Formula adjusted, standardized to suit performance parameters

Directions

Surfaces : Loosen cap and remove the HiDip slide from container. Aseptically remove the protective plastic cover from slide by taking care not to touch agar surfaces. Check for dehydration or contamination. Gently lower the slide and press agar to touch the test surface by bending the scull around the hinge line. Apply even and firm pressure for few seconds. Take care not to smudge agar over the test surface. Repeat procedure using the second agar surface on an area adjacent to the initial test side. Return the slide to the container and close tightly. Incubate in an up right position at indicated temperature.

Liquids : Loosen cap and remove the HiDip slide from the container. Aseptically remove the protective plastic cover from slide by taking care not to touch agar surfaces. Check for dehydration or contamination. Dip slide into test fluid so that agar surface becomes totally covered. (In case of inadequate liquid sample availability, pour sample over the surface of the slide). Allow to drain. Return the slide to the container and close tightly. Incubate in an upright position at indicated temperature. Label the container for sample number, source, date and time etc. for reference.

Disposal : Used HiDip slides should be handled carefully, as it contains live microorganisms. These slides can be best disposed off either by or by immersing in a suitable disinfectant solution (i.e. dettol, phenyl etc.) over night or by autoclaving them after loosening the cap. An autoclave is not essential, a domestic pressure cooker will suffice.

Principle And Interpretation

Plate Count Agar is formulated as described by Buchbinder et al (1) which is recommended by APHA (2,3,4) and FDA (5).

Casein enzymic hydrolysate provides amino acids and other complex nitrogenous substances. Yeast extract supplies Vitamin B complex. APHA recommends the use of pour plate technique. The samples are diluted and appropriate dilutions are added in Petri plates. Sterile molten agar is added to these plates and plates are rotated gently to ensure uniform mixing of the sample with agar. The poured plate count method is preferred to the surface inoculation method, since it gives higher results. Plate Count Agar is also suitable for enumerating bacterial count of sterile rooms.

Rose Bengal Chloramphenicol Agar was formulated originally by Jarvis 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 or papaic digest of soyabean meal provides essential growth nutrients. Dextrose 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 mould colonies such as *Rhizopus* species (4). The medium has neutral pH, which with the antibiotics has noted to be advantageous (5, 6). Rose bengal is taken up by mould and yeast colonies thereby assist in enumeration .

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 photodegradation of rose bengal yields compounds that are toxic to fungi (7, 8).

These present formulations incorporate neutralizing substances neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde. (1).

Sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurials; sodium thiosulfate neutralizes iodine and chlorine (1); lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface-active agent, neutralizes substituted phenolics (2-5). The disinfectants used for testing are 2% chlorine, 2% formaldehyde, 1% glutaraldehyde, 2% iodine, 2% phenol, 1/750 quaternary ammonium compounds, 1/1000 mercurials etc.

Quality Control

Appearance

The HiDip slide containing combination of sterile PCA Medium w/ TTC & Neutralizer and Rose Bengal Medium w/ Neutralizer on separate individual surfaces.

Colour

Light yellow coloured medium and Deep pink coloured medium

Quantity of medium

2.5ml of medium per surface

pH of PCA medium w/ TTC & Neutralizer

6.80- 7.20

pH of Rose Bengal medium w/Neutralizer

7.00- 7.40

Sterility test

Passes release criteria

Cultural response

Cultural characteristics observed after incubation at 35-37°C on PCA medium w/TTC & Neutralizer for 18-24 hours and at 20-25°C on Rose Bengal medium w/Neutraliz for 48-72 hours

Organism	Growth onMedium	Colour of Colony
Part A: PCA Medium w/TTC & Neutralizer		
<i>Escherichia coli</i> ATCC25922	Luxuriant	Red to maroon
<i>Lactobacillus casei</i> ATCC 9595	Luxuriant	Red to maroon
<i>S. serotype Enteritidis</i> ATCC 13076	Luxuriant	Red to maroon
<i>Bacillus subtilis</i> ATCC 6633	Luxuriant	Red to maroon
<i>Enterobacter aerogenes</i> ATCC 13048	Luxuriant	Red to maroon
<i>Enterococcus faecalis</i> ATCC 29212	Luxuriant	Red to maroon
<i>Staphylococcus aureus</i> ATCC 25923	Luxuriant	Red to maroon
<i>Streptococcus pyogenes</i> ATCC 19615	Luxuriant	Red to maroon
Part B: Rose Bengal medium w/Neutralizer		
<i>Aspergillus niger</i> ATCC 16404	Good	
<i>Candida albicans</i> ATCC 10231	Good	Cream to off white
<i>Micrococcus luteus</i> ATCC 10240	Inhibited	
<i>Saccharomyces cerevisiae</i> ATCC 9763	Good	Cream to off white

Storage and Shelf Life

Store between 5-25°C. Use before expiry date on the label.

Reference

Refer Technical Data of M091 Plate count agar & M640 Rose Bengal chloramphenicol agar .

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Disclaimer :

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