TECHNICAL DATA SHEET

DICHLORAN ROSE BENGAL CHLORAMPHENICOL (DRBC) AGAR

ENUMERATION OF YEASTS & MOLDS

1 INTENDED USE

Dichloran Rose Bengal Chloramphenicol agar is recommended for the enumeration of viable yeasts and molds that develop in products destined for human and animal consumption with a water activity (a_w) greater than 0.95.
The media does not allow for the enumeration of mold spores.

The typical composition of the media corresponds to that defined in the standard NF EN ISO 21527-1.

2 HISTORY

The first selective media destined for the enumeration of yeasts and molds used an acid pH to limit the proliferation of bacteria. In 1962, work performed by Mossel on yeast and mold detection in foods showed the superiority of media at a more neutral pH and containing an antibacterial agent over the system based on acid pH alone. In 1973, Jarvis developed and used with success Rose Bengal Chlortetracycline. During the course of subsequent work in 1979, King et al. showed that the introduction of dichloran and the reduction of the concentration of rose bengal allowed a greater recovery of molds. The results obtained by these same authors also demonstrated that sporulation was better controlled at a pH of 5.6 on this media. Similarly in 1978, Korburger and Rogers showed the efficiency of the combination of chloramphenicol and chlortetracycline for bacterial inhibition. Finally, in 1992, based on the comparative studies of detection and enumeration, Beuchat proposed that a surface inoculation be favored over a pour plate method for enumeration of yeasts & molds.

3 PRINCIPLES

The peptones and glucose assure the growth of the yeasts and molds.
Dichloran and rose bengal inhibit the invasion of molds and reduces the size of other colonies.
Rose bengal is easily assimilated by yeasts which tend to facilitate their counting by coloring them pink.

The presence of chloramphenicol, a heat resistant antibiotic, and chlortetracycline reinforces the selectivity of the medium against the majority of bacterial contaminants. Tergitol limits proliferation by *Mucoraceae*.

Zinc and copper in the form of sulfates improve the pigment production by molds.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Polypeptone ..... 5,0 g
- Glucose ..... 10,0 g
- Monopotassium phosphate ..... 1,0 g
- $MgSO_4,H_2O$ ..... 0,5 g
- Dichloran ..... 2,0 mg
- Rose bengal ..... 25,0 mg
- Chloramphenicol ..... 50,0 mg
- Chlortetracycline chlorhydrate ..... 50,0 mg
- $ZnSO_4,7H_2O$ ..... 10,0 mg
- $CuSO_4,5H_2O$ ..... 5,0 mg
- Tergitol ..... 1 mL
- Bacteriological agar ..... 12,4 g

pH of the ready-to-use media at 25 °C : 5,6 ± 0,2.

5 PREPARATION

- Suspend 30.0 g of dehydrated media (BK198) in 1 liter of distilled or demineralized water.
- Slowly bring to a boil, stirring with constant agitation until complete dissolution.
- Dispense 100 mL into vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool the molten media to 44-47 °C.
- Pour into sterile Petri plates.
- Let solidify on a cold, flat surface.

✓ **Reconstitution :**
30,0 g/L

✓ **Sterilization :**
15 min at 121 °C

Use of the ready-to melt media :

- Melt the media (if it was prepared in advance) or the ready-to melt media (BM142) for the least amount of time needed to achieve total liquefaction.
- Cool and maintain at 44-47 °C.

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator, with the covers partially removed.
- Transfer 0.1 mL of the product to analyze and its serial tenfold dilutions to these prepared plates.
- Spread the inoculum evenly on the surface using a sterile triangle or “hockey stick”.
- Incubate the plates, cover on top, at 25 ± 1 °C for 2 to 5 days.

✓ **Inoculation :**
0,1 mL on the surface

✓ **Incubation :**
2 to 5 days at 25 ± 1 °C

Notes :

When using the technique of inoculation by inclusion (in depth), the equivalency of the results should be validated against that of surface.

Cytotoxic decomposition products caused by exposure to light can result in underestimation of the mycoflora present in the sample. It is therefore highly recommended to avoid light exposure during incubation.

7 RESULTS

Only retain those plates containing less than 150 colony forming or mycelial forming units.
If rapid invasion of the plates is observed, retain the counts made after 2 days, then again after 5 days of incubation.
Yeasts appear pinkish due to assimilation of the rose bengal.

8 QUALITY CONTROL

Dehydrated media : cream to pinkish powder, free-flowing and homogeneous.
Prepared media : pink agar.

Typical culture response after 5 days of incubation at 25°C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
Saccharomyces cerevisiae	WDCM 00058	$P_R \geq 50$ %
Aspergillus brasiliensis	WDCM 00053	$P_R \geq 50$ %
Escherichia coli	WDCM 00013	Inhibited, score 0
Bacillus subtilis ssp. *spizizenii*	WDCM 00003	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated media : 2-20 °C.
Ready-to-melt media : 2-8 °C.
The expiration dates are indicated on the label.

Prepared media in vials (*) : 90 days at 2-8 °C.
Prepared media in plates (*) : 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer’s instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK198HA

Ready-to-melt media :
10 x 200 mL vials BM14208

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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