



Dichloran Medium Base w/ Rose Bengal

M1000

Intended Use:

Recommended for selective isolation and enumeration of yeasts and moulds associated with food spoilage.

Composition**

| Ingredients | Gms / Litre |
|--------------------------------|-------------|
| Peptone | 5.000 |
| Dextrose (Glucose) | 10.000 |
| Potassium dihydrogen phosphate | 1.000 |
| Magnesium sulphate | 0.500 |
| Rose bengal | 0.025 |
| Dichloran | 0.002 |
| Agar | 15.000 |
| Final pH (at 25°C) | 5.6±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.76 grams in 500 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 and aseptically add sterile reconstituted contents of 1 vial of Chloramphenicol Selective Supplement (FD033). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dichloran Medium Base with Rose Bengal is formulated as described by King et al (5), which is a modification of Rose Bengal Chloramphenicol Agar (3). Dichloran Medium Base with Rose Bengal is used along with Rose Bengal Chloramphenicol Agar where it is necessary to enumerate yeasts in the presence of moulds.

Peptone provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Rose bengal exhibits an improved inhibitory activity at pH 5.6 and therefore the final pH of the medium is reduced to 5.6 for the inhibition of spreading fungi (5). The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing moulds. The concentration of rose bengal is reduced for optimal performance with dichloran. Additionally, rose bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated.

Add 40 ml of food sample to 200 ml of 0.1% Peptone water (M028) and shake periodically for 30 minutes (7) or process in stomacher for 30 seconds (8). Inoculate 0.1 ml of this sample on Dichloran Medium Base with Rose Bengal. Report the number of colonies per gram of food.

Type of specimen

Food sample : Eggs, Meat, Dairy products (except milk powder), Fruits, Vegetables, Fresh pastes, etc.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,9).

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. This medium is general purpose medium and may not support the growth of fastidious organisms.

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

Pink coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.15% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed with added Chloramphenicol Selective Supplement (FD033), after an incubation at 25-30°C for upto 6 days.

| Organism | Inoculum (CFU) | Growth | Recovery |
|---------------------------------------------------------------|------------------|----------------|----------|
| <i>Bacillus subtilis subsp. spizizenii</i> ATCC 6633 (00003*) | ≥10 ⁴ | inhibited | 0% |
| <i>Candida albicans</i> ATCC 10231 (00054*) | 50-100 | good-luxuriant | ≥50% |
| <i>Escherichia coli</i> ATCC 25922 (00013*) | ≥10 ⁴ | inhibited | 0% |
| <i>Mucor racemosus</i> ATCC 42647 (00181*) | 50-100 | good-luxuriant | |
| <i>Saccharomyces cerevisiae</i> ATCC 9763 (00058*) | 50-100 | good-luxuriant | ≥50% |

Key : * - Corresponding WDCM numbers

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. 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 (2,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. Jarvis B., 1973, J. Appl. Bact., 36:723.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. King D.A. Jr., Hocking A.D. and Pitt J.I., 1979, J. Appl. Environ. Microbiol., 37:959.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
7. Sharf J.M. (Ed.), 1966, American Public Health Association, 2nd ed., New York.
8. Sharp A.N. and Jackson A.K., 1972, J. Appl. Bact., 24:175.
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 02/ 2019

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