Chloramphenicol Yeast Glucose Agar

M1008

Intended Use

Recommended for selective enumeration of yeasts and moulds in milk and milk products.

Composition**

Ingredients	g / L
Yeast extract	5.000
Dextrose (Glucose)	20.000
Chloramphenicol	0.100
Agar	14.900
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Chloramphenicol Yeast Glucose Agar is a selective medium recommended for isolation and enumeration of fungi-yeasts and moulds in milk and milk products (1,2,3). Recently this medium has been recommended by ISO committee for the enumeration of yeasts and moulds (4). The medium contains yeast extract, which provides nitrogenous nutrients and vitamin B complex. Dextrose is the energy source. Chloramphenicol, a thermostable antibiotic, suppresses accompanying bacterial flora. This improves shelf life of the prepared medium and the prepared medium can be used over a period of at least 4 months (5).

Technique: Take two sterile Petri plates and transfer 1 ml of sample (if liquid) or 1 ml of the initial suspension in case of other products. Further take another two sterile plates and transfer 1 ml of 10-1 dilution to each sterile Petri plate or 1 ml of 10-2 dilution for other products. Repeat the procedure using further dilutions if necessary. Pour about 15 ml of Chloramphenicol Yeast Glucose Agar (5) previously melted and maintained at 45 ± 1°C. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes shall not exceed 15 minutes. Carefully mix the inoculum with the medium and allow it to solidify. Prepare control plate to check the sterility. Incubate the plates at 25± 1°C. Count the colonies on each plate after 3, 4 and 5 days incubation. It is necessary to carry out a microscopic examination in order to distinguish, according to their morphology, the colonies of yeast and moulds from colonies of bacteria.

It is advisable to examine the plates at the end of three days for yeast colonies, as they are likely to be overgrown by moulds growth. Make a separate count of yeast colonies, which are characterized, as smooth, moist, elevated surface colonies. Count moulds colonies, which are recognized by their profuse growth of hyphae. If only yeast counts are required, add 0.25% of sterile sodium propionate solution to the medium at the time of preparation of plates to inhibit the growth of moulds (6).

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9).
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. Further biochemical tests must be carried for further confirmation.

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
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.49% Agar gel.

Colour and Clarity of prepared medium
Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH
6.40-6.80

Cultural Response
Cultural characteristics observed after an incubation at 22-25°C for 2-5 days.

Organism	Inoculum (CFU)	Growth	Recovery
# *Aspergillus brasiliensis* ATCC 16404 (00053*)	50-100	good-luxuriant	
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good-luxuriant	>=50%
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%

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

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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3. International Organization for Standardization (ISO), Draft ISO/DIS 6611.
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6. International Organization for Standardization (ISO), 1999, ISO 5403 :1999.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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