Sabouraud Dextrose Agar, Granulated

GMH063

Intended Use

Recommended for the cultivation of yeasts, moulds and aciduric bacteria from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	40.000
Mixture of Peptone and Tryptone (1:1)##	10.000
Agar	15.000
pH after sterilization(at 25°C)	5.6±0.2

Mixture of Peptic digest of animal tissue and Pancreatic digest of casein (1:1)#

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates or as desired.

Principle And Interpretation

Fungi were among the first microorganisms recognized because some of the fruiting structures, such as the mushrooms, are large enough to be seen without a microscope. Fungi can be grouped simply on the basis of morphology as either yeasts or moulds (1). Fungal diseases that occur on the skin, hair and mucous membrane are called superficial mycoses, and the organism that cause them, the dermatophytes (2). Where fungi are to be isolated, it is good practice to use a medium that favors their growth but is not optimal for the growth of bacteria.

Sabouraud Dextrose Agar is Carliers modification (3) of the formulation described by Sabouraud (4) for the cultivation of fungi (yeasts, moulds), and aciduric microorganisms. Sabouraud Dextrose Agar is recommended for microbiological examination of non-sterile products in accordance with the harmonized method of USP/EP/BP/JP (5-8). This medium is also employed in microbial limit tests in pharmaceutical testing, food and cosmetics (9)

Peptone and Tryptone provides carbonaceous, nitrogenous compounds, long chain amino acids, vitamins and other essential growth nutrients. Dextrose (Glucose) provides an energy source. High dextrose concentration and low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (10).

Some pathogenic fungi may produce infective spores, which are easily dispersed in air, so examination should be carried out in safety cabinet. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth. Growth of white colonies may be indicative of presence of *Candida albicans.* The total combined yeast and molds count is considered to be equal to the number of colony forming unit found using this medium, if bacterial colonies are detected they are counted as part of total yeast and mold count. In case the bacterial colonies exceeds the acceptance criterion, then antibiotics can be supplemented in this medium

Type of specimen

Pharmaceutical samples;

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (5-8).

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 handing specimens and culture. Standard guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. For heavily contaminated samples, the media must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.
2. Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet.

3. Further biochemical tests should be carried out for 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 colored granular medium

Gelling
Firm, comparable with 1.5% Agar gel

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

pH
5.40-5.80

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP), after an incubation at 30-35 °C for 24-48 hours.Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar

Growth Promoting Properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <= 100 cfu (at 30-35°C for <=24 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 24-48 hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth Promotion + Indicative						
Candida albicans ATCC 10231 (00054*)	50 -100	Luxuriant (white colonies)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Growth Promotion + Total yeast and mould count						
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant	35 -100	>=70 %	20 -25 °C	<=5 d
#Aspergillus brasiliensis ATCC 16404 (00053*)	50 -100	luxuriant	35 -100	>=70 %	20 -25 °C	<=5 d
Additional Microbiological Testing						
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant	35 -100	>=70%	30 -35 °C	24 -48 hrs
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	good(inhibited on media with low pH)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	good(inhibited on media with low pH)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Escherichia coli NCTC 9002	50 -100	good(inhibited on media with low pH)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Trichophyton rubrum ATCC 28191	50-100	good			20 -25 °C	<=5 d

Lactobacillus casei ATCC 334	50 -100	luxuriant	35 -100	>=70 %	30 -35 °C	24 -48 hrs

Key : (#) - Formerly known as *Aspergillus niger,* (*) - corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on thelabel. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

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