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## TECHNICAL PRODUCT INFORMATION

### SABOURAUD DEXTROSE AGAR

#### INTENDED USE:

This medium has been long used in Microbiology for the cultivation of fungi, especially Dermatophytes. The low pH of approximately 5.6 is beneficial for the growth of fungi and slightly inhibitory to contaminating bacteria in clinical specimens.

Contact plates are specifically designed to allow overflow of the medium which produces a meniscus or dome shaped surface. The plates can be pressed onto a surface for sampling the microbial bioburden.

After sampling, the plates are covered and incubated at the appropriate temperature. The presence and number of organisms is determined by growth of colonies on the agar surface.

Collection of samples from the same area before and after cleaning and treatment with a disinfectant allows evaluation of the effectiveness of sanitary procedures. Contact plates are useful for monitoring surfaces in clean rooms and other environmentally controlled areas. Additionally, contact plates are recommended for use in air sampling equipment. Contact plates are for laboratory use only.<sup>3</sup>

Northeast Laboratory Services offers a wide range of Sabouraud Dextrose Agar available in petri dish, tube and bottle. Consult the NEL Catalog of Products or call for more information.

#### HISTORY/SUMMARY:

In 1982 Sabouraud described a suitable formulation for the cultivation of fungi<sup>1</sup>. Since then, numerous modifications of his original media have been made. George and colleagues<sup>2</sup> found the addition of antibiotics such as Penicillin, Streptomycin and Cycloheximide to Sabouraud media greatly enhanced the recovery of fungi from heavily contaminated specimens.

Emmans<sup>5</sup> modified the original formulation of Sabouraud, by reducing the concentration of Dextrose to 2% and changing the pH of the medium by near neutral (pH 6.9). His formulation is preferred if antibiotics are added in various concentrations to further inhibit growth of bacteria and saprophytic fungi.

#### PRINCIPLES:

The medium contains Dextrose as an energy source and peptones as nitrogen and vitamin sources to support the growth of most fungi. Agar is the solidifying agent. The low pH of Sabouraud inhibits bacteria growth. Antibiotics may be added in various concentrations to further inhibit growth of bacteria and saprophytic fungi.

The addition of Lecithin and Polysorbate 80 is utilized to neutralize antiseptics and disinfectants for environmental monitoring and other manufacture applications.

Lecithin neutralizes quaternary ammonium compounds and ethanol. Polysorbate 80 neutralizes phenols, hexachlorophene and formalin.

Complete neutralization of disinfectants is important, as carryover of disinfectants can cause false no-growth test results.<sup>3</sup>

**FORMULA:**

SDA	
Component per liter of purified water	Amount
Casein/Meat peptone	10 g
Dextrose	40 g
Agar	15 g

Final pH: 5.6 ± 0.2 @ 25°C

SDA w/ Lecithin & Polysorbate 80	
Component per liter of purified water	Amount
Sabouraud Dextrose Agar	65.0 g
Lecithin	0.7 g
Polysorbate 80	5.0 mL
Agar	5.0 g

Final pH: 5.6 ± 0.2 @ 25°C

**PRECAUTIONS:**

Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director. These products are for In Vitro Diagnostic Use or Laboratory Use Only as indicated by product label.

**STORAGE:**

Store media at 2-8°C, adequate storage prolongs the life and quality of the product. Use media prior to expiration date.

**SPECIMEN COLLECTION:**

The success in recovering suspected pathogens from a specimen greatly depend on a number of factors such as site chosen for collection, media and environmental conditions used for incubation, temperature, inoculum size used, etc. Standard procedure should be chosen and these procedures should be implemented by suitably trained personnel under the supervision of a microbiologist or other qualified personnel.

**PROCEDURE:****15 X 100 mm Monoplate:**

1. Allow media to warm to room temperature before use.
2. Using appropriate protocol, media is inoculated with specimen and incubated.
3. The temperature choice is an important factor in fungus recovery.
  - a. Molds grow best at 25 - 30°C.
  - b. Yeasts are generally cultivated at 37°C.
  - c. Most systemic fungi as well as other species of fungi exhibit dimorphism and conversion from yeast phase (37°C) to mold phase (35°C) and vice versa is necessary to establish identity of some species.
  - d. Therefore it is useful to inoculate media in duplicate and incubate at different temperatures so proper recovery of fungi is assured.
  - e. Aerosols of fungal spores during handling of these cultures may be dangerous to the worker. Handling of mycological specimens should be performed under a properly functioning safety cabinet.
4. Follow laboratory procedure for isolation and identification of organism growth.

**Contact Plate:**

1. Samples may be collected in duplicate.
2. Select plate and carefully remove lid.
3. Hold the plate with thumb and second finger:
  - a. Use the index finger to gently roll plate edge against sampling site, then gently press plate center to ensure all agar has contacted the sample surface.
  - b. Do not move agar plate side to side to avoid spreading contaminants over the agar surface.
  - c. Moving side to side impedes individual growth of colony formation, by causing confluent growth of colonies.
4. Incubate exposed plates at 35 ± 2°C for 48 hours and 25 ± 2 °C for 7 days or as required.

5. After incubation:
  - a. Count the colonies with distinct margins
  - b. Count all visible colonies
    - i. Spreading colonies should be counted as one
    - ii. Care should be taken to observe other distinct colonies intermingled in the growth around the periphery or along a hairline
    - iii. Count intermingled colony, bicolored colony or halo-type spreaders each as one colony
  - c. Generally 200 colonies is the maximum that can be counted on each plate
  - d. Record the number of colonies counted
6. Subculture colonies of interest to selective mediums for identification by biochemical testing and/or microscopic examination of smears such as a gram stain.

**LIMITATIONS:**

The ability to detect yeast, molds and fungi by culture techniques can be affected by improper specimen collection, storage and inoculation, improper incubation temperatures or atmospheres, improper incubation time and improper storage and handling of media.

In addition, contact plates are intended for the enumeration of microorganisms on surfaces of sanitary importance. For identification, the organism must be in pure culture form. Biochemical tests may be performed for complete identification.

Follow your standard procedures for organism work up. Consult appropriate references or texts as necessary.

**PERFORMANCE CHARACTERISTICS:**

Approval by NEL of each lot of Sabouraud Dextrose Agar is based on results obtained on a number of tests, among them bacteriological performance with known microorganisms.

Organisms	Results
Candida albicans	Large creamy white colonies
Candida other than albicans	Large white colonies
Saccharomyces cerevisiae	Large creamy white colonies
Aspergillus	Good growth – spores by 7 days
Penicillin species	Good growth – spores by 7 days
Cryptococcus neoformans	Good growth – mucoid colonies
Trichophyton	Fair growth – white fluffy colonies

**QUALITY CONTROL:**

It is recommended that the laboratory confirm the performance characteristics of this media. Careful selection of test organisms must be made so maximum information on the product suitability is obtained.<sup>6</sup>

**REFERENCES:**

1. Sabouraud, R. 1892. Ann. Dermatol.Syphilol.3:1061
2. Science; 114:387, 1951
3. Difco & BBL Manual, 2003
4. Bailey & Scott's Diagnostic Microbiology, 12<sup>th</sup> Edition. Mosby Elsevier 2007
5. Diagnostic Procedures and Reagents, 4<sup>th</sup> ed., PAHA, Inc., NY 1963
6. M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard-3<sup>rd</sup> Edition June 2004 (CLSI – Clinical and Laboratory Standards Institute)
7. Manual Clinical Microbiology. ASM, 2<sup>nd</sup> edition, 1974