Dehydrated Culture Media

SABOURAUD DEXTROSE AGAR

Code: CM0041

an acidic pH medium for the isolation of dermatophytes, other fungi and yeasts

Typical Formula*

	gm/litre
Mycological peptone	10.0
Glucose (dextrose)	40.0
Agar	15.0

pH 5.6 ± 0.2 @ 25°C

*** Adjusted as required to meet performance standards**

Directions

Add 65g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour in to sterile Petri dishes.

Description

This modification of Sabouraud agar (Carlier[1]) is suitable for the cultivation and differentiation of fungi.

Carlier showed that the medium gives reliable results with *Microsporum audouini, Microsporum canis, Trichophyton mentagrophytes, Trichophyton flavum, Trichophyton rubrum* and *Candida albicans*.

Sabouraud Dextrose Agar may be used in place of the Standard American medium of Hodges[2]. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud[3].

The medium is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria.

Georg *et al*.[4] aseptically added 0.5g cycloheximide, 20,000 units penicillin and 40,000 units streptomycin to each litre of autoclaved, cooled medium. *Cryptococcus neoformans, Aspergillus fumigatus* and *Allescheria boydii* are sensitive to cycloheximide; *Actinomyces bovis* and *Nocardia asteroides* are sensitive to penicillin and streptomycin.Alternatively, one may add 0.4g chloramphenicol and 0.05g cycloheximide to each litre of reconstituted medium before autoclaving (Ajello[5]). The same micro-organisms are sensitive to this new combination - see Dermasel Selective Supplement SR0075.

Williams Smith & Jones[6] employed Oxoid Sabouraud Dextrose Agar, containing 20,000 units penicillin and 0.04g neomycin per litre, for the count of yeasts in the alimentary tract of the pig. Hantschke[7] used colistin, novobiocin and cycloheximide to isolate *Candida albicans*. Dolan[8] used gentamicin, chloramphenicol and cycloheximide for the selective isolation of pathogenic fungi.

Oxoid Sabouraud Dextrose Agar may also be used as the basis of a Pagano-Levin medium[9] for the isolation of *Candida albicans*. 0.1g of triphenyltetrazolium chloride (as a filter sterilised solution) is added to each litre of autoclaved molten medium cooled to 55°C. The medium is usually made inhibitory to most non-pathogenic fungi and bacteria by the addition of antibiotics as above. After incubation for 3 days at 25°C, *Candida albicans* colonies are unpigmented or pale pink whilst other *Candida* species and other fungi form deeper pink or red colonies. The test is adequate for screening purposes but other diagnostic criteria should also be utilised for the identification of *Candida albicans*[10,11,12,13]. The quality control of Oxoid Sabouraud Dextrose Agar includes testing in accordance with ISO:11133 2014[14].

Technique

1. Inoculate each specimen in duplicate.
2. Incubate one set of media aerobically at 22-25°C and the other set at 35°C for 5-30 days. Loosen the caps of tubes and ensure adequate moisture for the plates to compensate for loss of water vapour. **DO NOT SEAL THE PLATES**.
3. Examine every 2-4 days.
4. Describe each specific type of colony morphology and sub-culture to appropriate media for further identification tests.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.
Prepared medium: Light straw to straw coloured gel.

Quality Control

Positive controls:
Expected results
Candida albicans ATCC® 10231*
WDCM 00054
Good growth; cream colonies
Aspergillus brasiliensis ATCC® 16404 *
WDCM 00053
White mycelium; black spores
*Saccharomyces cerevisiae ATCC® 9763**
WDCM 00058
Good growth; cream domed colonies
Negative control:

Uninoculated medium
No change

* This organism is available as a Culti-Loop®

Precautions
Some of the pathogenic fungi may produce infective spores which are are easily dispersed into the laboratory. Such organisms should be examined only within a protective cabinet.

The combination of cycloheximide and chloramphenicol inhibits many pathogenic fungi[4]. However, the mycelial phase of *Histoplasma capsulatum, Paracoccidioides brasiliensis, Sporothrix schoenckii* and *Blastomyces dermatitidis* is not inhibited by these antibiotics when incubated at 25-30°C [15].

Please check relevant health and safety documentation before working with cyclohexamide.

References

1. Carlier Gwendoline I. M. (1948) *Brit. J. Derm. Syph*. 60. 61-63.
2. Hodges R. S. (1928) *Arch. Derm. Syph., New York*, 18. 852.
3. Sabouraud R. (1910) `*Les Teignes', Masson, Paris*.
4. Georg Lucille K., Ajello L. and Papageorge Calomira (1954) *J. Lab. Clin. Med*. 44. 422-428.
5. Ajello Libero (1957) *J. Chron. Dis*. 5. 545-551.
6. Williams Smith H. and Jones J. E. T. (1963) *J. Path. Bact*. 86. 387-412.
7. Hantschke D. (1968) *Mykosen.* 11. 113-115.
8. Dolan C. T. (1971) *Appl. Microbiol*. 21. 195-197.
9. Pagano J., Levin J. G. and Trejo W. (1957-58) *Antibiotics Annual* 1957-58, 137-143.
10. Kutscher A. H., Seguin L., Zegarelli E. V., Rankow R. M., Mercadante J. and Piro J. D. (1959a) *J. Invest. Derm*. 33. 41-47.
11. Kutscher A. H., Seguin L., Zegarelli E. V., Rankow R. M., Campbell J. B. and Mercadante J. (1959b) *Antibiotics and Chemotherapy* 9. 649-659.
12. Sinski J. T. (1960) *J. Invest Dermat*. 35. 131-133.
13. Ridley M. F. (1960) *Australian J. Dermat*. 5. 209-213.
14. ISO 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media
15. McDonough E. S., Georg L. K., Ajello L. and Brinkman S. (1960) *Mycopath. Mycol. Appl*. 13. 113-116.