INSTRUCTIONS FOR USE

.

SABOURAUD DEXTROSE AGAR

Dehydrated culture medium

Sabouraud Dextrose Agar: *Candida albicans*

1-INTENDED USE

In vitro diagnostic. General purposes medium for the isolation and cultivation of yeasts and moulds, from clinical and non-clinical specimens.

2-COMPOSITION - TYPICAL FORMULATION *

Pancreatic digest of casein	5 g
Peptic digest of meat	5 g
Glucose	40 g
Agar	15 g
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3-PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The Italian lawyer and farmer Agostino Bassi in 1835 discovered the mycotic nature of an epidemic disease of silkworms called muscardine; this recognition of the relationship between fungi and disease was the basis for the development of medical mycology.[1] By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for the their isolation and classification.[1,2]
Numerous experiments were performed to improve Sabouraud's formula with a variety of peptones and carbohydrates by Weidman and Spring[3] but the suitable medium was selected by Hodges[4]. In its final formulation, this medium contained a 1% peptone, 4% dextrose, and 1.8% agar, with a final pH of 5.0. This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories.[1]
Sabouraud Dextrose Agar is a non-selective medium for the isolation and cultivation of yeasts and moulds, especially dermatophytes, from clinical specimens[5-7]. It is recommended for the total combined yeasts and moulds count and for the detection of *C.albicans* in non-sterile pharmaceutical products according to the harmonized EP, USP, JP method[8].
Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria. Glucose, at high concentration is a carbon and energy source.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 65 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes.
Notes
Do not exceed the boiling and sterilization times and temperatures.
Alternatively distribute in screw capped tubes before sterilization and solidify in slanted position.

5-PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5,6 ± 0.2

6-MATERIALS PROVIDED

Product	Type	REF	Pack
Sabouraud Dextrose Agar	Dehydrated medium	4020052	500 g (7,7 L) CND: W0104030101; EDMA 14.03.01.01; RDM: 1875069/R
		4020054	5 kg (77 L) CND: W0104030101; EDMA 14.03.01.01; RDM: 1875072/R

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8-SPECIMENS

Sabouraud Dextrose Agar can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[5-7] Sabouraud Dextrose Agar is not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[5] For pharmaceutical samples, refer to the EP for details on sample collection and preparation.[8]

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9-TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
Inoculate each specimen in duplicate; incubate one set in aerobic condition at 22-25°C, the other at 33-37°C.[10]
For dermatophytes, examine cultures every 4-6 days for a period of up to 20 days; for others incubate 2-5 days. Plates should be incubated under conditions of increased humidity during prolonged incubation.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

For the detection of *C.albicans* in non-sterile pharmaceuticals products, the technique recommended by European Pharmacopoeia[8] and summarized below should be followed:

- Prepare a sample suspension in 100 mL of Sabouraud Broth using 10 mL of sample or the quantity corresponding to not less than 1 g of or 1 mL of the product to be examined. Mix and incubate at 30-35°C for 3-5 days.
- Subculture on a plate of Sabouraud Dextrose Agar and incubate at 30-35 °C for 24-48 hours.

10-READING AND INTERPRETATION

Clinical specimen: after incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.
Non-sterile pharmaceutical products: growth of white colonies indicates the possible presence of *C.albicans*; this is confirmed by identification tests. The test is to be considered negative if such colonies are not present or if the identification tests are negative.

11-USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C.albicans ATCC 10231	25-35°C / up to 72 h / A	good growth, white yeast-like colonies
T.mentagrophytes ATCC 9533	25-35°C / up to 72 h / A	good growth, white colonies with typical morphology

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12-PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Sabouraud Dextrose Agar (Test Batch-TB) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative test with the target strains *C.albicans* ATCC 10231*, A.brasiliensis* ATCC 16404, *S.cerevisiae* ATCC 9763; Sabouraud Dextrose Agar plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 30-35°C and at 20-25°C for 24 and 72 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr = CFU_{TB}/CFU_{RB}$) is calculated. If *Pr* is ≥ 0,7 and if the colonies morphology is typical, the results are considered acceptable and conform to the specifications.
Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following strains *P.chrysogenum* ATCC 10106, *T.mentagrophytes* ATCC 9533*, M.canis* ATCC 36299*. A*fter incubation at 20-25°C for up to 72 hours, the amount of growth on the plates and colonies' characteristics are evaluated and recorded: they shall be comparable in both batches.
The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-6} of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922 and *S.aureus* ATCC 25923. The growth of non-target strain is partially inhibited in both batches.

13-LIMITATIONS OF THE METHOD

- Sabouraud Dextrose Agar has poor selective properties; a selective medium should be inoculated in parallel for isolation of fungi from potentially contaminated specimens.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the preparation process of plated or tubed or bottled media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.

- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (plates/tubes/bottles), and the storage method applied (temperature and packaging).

16 - REFERENCES

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5. McGowan K. Specimen Collection, Transport and Processing: Mycology. *In* Jorgensen JH, Pfaller et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; Vol.2 2015.
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7. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
8. European Pharmacopoeia, current edition
9. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05
Revision 2	Modification of "precautions and warnings", "storage conditions and shelf life".	2022/01

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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