SABOURAUD DEXTROSE AGAR

Ready-to-use plates

Sabouraud Dextrose Agar: *Candida albicans*

1 - INTENDED USE

In vitro diagnostic device. 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 slightly inhibitory to contaminating bacteria. Glucose, at high concentration is a carbon and energy source.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	yellow, limpid
Final pH at 25 °C	5.6 ± 0.2

5 - MATERIALS PROVIDED

Product	Type	REF	Pack
Sabouraud Dextrose Agar	Ready-to-use plates	542005	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Sabouraud Dextrose Agar can be directly inoculated with many clinical specimens such as skin, hair, nails, genital, respiratory and urine samples and non-clinical specimens. Refer to the quoted literature for specimens' 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]

8 - 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, 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.

9 - 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.

10 - 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, the end user can perform its own Quality Control 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

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to use plates of Sabouraud Dextrose Agar and of the raw material used for the production of prepared plates, dehydrated Sabouraud Dextrose Agar REF 402005, (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. After 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.
Accuracy was assessed by reviewing the Quality Control data. The results of 45 batches produced from 1/1/2019 to 25/5/2020 were evaluated. 100% of the batches showed conformity to defined acceptance criteria in terms of productivity and morphological and chromatic characteristics with target strains.

12 - LIMITATIONS OF THE METHOD

- Sabouraud Dextrose Agar has poor selective properties; combined workflows with sample inoculated on SDA, chromogenic media and selective media are recommended to achieve optimal sensitivity, specificity and speed of analysis.
- Growth on the medium depends on the metabolic requirements of each microorganism; some target strains may not be able to grow or may show a delayed growth. A lack of growth or the absence of typical colonies does not preclude the presence of yeasts and moulds in the sample.
- The device is not intended to diagnose infections or to guide the antimicrobial therapy. It is used in a diagnostic set of investigations to provide microbial colonies isolated from clinical samples of patients with suspected fungal infection. Appropriate tests are required for complete identification and epidemiological typing of colonies; if necessary, perform antimicrobial susceptibility tests using recommended methods.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic device intended for professional use only, is not automated and is not a companion diagnostic tool. It must be used by adequately trained and qualified laboratory personnel, observing biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. Therefore, it is recommended that the ready-to use plates 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 for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal and dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains, in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify the Manufacturer (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- The Manufacturer may not be held responsible for any loss or damage in any way resulting from or related to use of the product in manners not compliant with the instructions provided.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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3. Weidman FD, Spring D. Comparison of ringworm culture ingredients: II and III. Arch Dermatol Syphilol 1928; 18:829–851.
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7. The Royal College of Pathologists. Bacteriology. https://www.rcpath.org/profession/publications/standards-for-microbiology-investigations/bacteriology.html
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 Catalogue number	LOT Batch code	IVD *In vitro* diagnostic medical device	Manufacturer	This way up	For single use only	CE European conformity mark
Temperature limitations	Contents sufficient for <n> tests	Consult electronic instructions for use	Use by	Keep away from light	Fragile, handle with care	UDI Unique device identifier

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Removal of obsolete classification	2023/03
Revision 3	Specimens, performances characteristics, limitation of the method, precautions and warnings, table of applicable symbols, references.	2025/11

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