

**INSTRUCTIONS FOR USE**

SABOURAUD BROTH

Dehydrated culture medium



Sabouraud Broth – from left: uninoculated tube and tube with *Aspergillus brasiliensis*

1 - INTENDED USE

In vitro diagnostic. Liquid medium for the cultivation of yeasts and moulds.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptic digest of animal tissue	5 g
Pancreatic digest of casein	5 g
Glucose	20 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sabouraud Broth, also known as Sabouraud Dextrose Broth, is a modification proposed by Sabouraud to the Sabouraud Dextrose Agar medium, without agar and with half-concentration of glucose.^{1,2}

Sabouraud Broth is a liquid medium intended for the cultivation of yeasts and moulds isolated from clinical or non-clinical specimens.³ The medium is recommended for growth promotion test, for the preparation of the sample and its pre-enrichment in the procedure for the detection of *Candida albicans* in non-sterile products with EP harmonized method.⁴ Sabouraud Broth complies with the quality specifications reported by European Pharmacopoeia.

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 aciduric microorganisms. Glucose, at high concentration is a carbon and energy source.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute in tubes or flasks and sterilize by autoclaving at 121°C for 15 minutes. Do not overheat.

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, PACKAGING

Product	Type	REF	Pack
Sabouraud Broth	Dehydrated medium	4020002	500 g (16,7 L)
		4020004	5 kg (167 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, ancillary culture media and reagents.

8 - SPECIMENS

Sabouraud Broth is mainly intended for the cultivation of yeasts and moulds isolated from clinical and non-clinical specimens. In pharmaceutical microbiology, the samples consist of non-sterile products on which to detect *C. albicans*. Refer to the European Pharmacopoeia for sample collection and transport procedures.⁴

9 - TEST PROCEDURE

Allow the tubes to reach room temperature.

General procedure

Inoculate each test strain or specimen into duplicate tubes. Incubate one tube at 22-25°C and the second at 35°C for 2-7 days.

The incubation conditions may vary according to the type of expected microorganisms and can be extended up to 30 days.

The user is responsible for choosing the appropriate incubation time, and temperature depending on the processed specimen or inoculated strain, the requirements of organisms to be recovered or cultivated and the local applicable protocols.

Detection of *C. albicans* in non-sterile pharmaceutical products.⁴

Prepare the sample suspension in 100 mL of Sabouraud Broth using at least 10 g or 10 mL of sample to be examined. Incubate this suspension at 30°C - 35°C for 3-5 days.

Using a loop, subculture from Sabouraud Broth onto Sabouraud Dextrose Agar plate (REF 402005) and incubate 30°C-35°C for 24-48 hours.

Growth of white colonies may indicate the presence of *C. albicans* to be confirmed by appropriate identification tests

The test is considered negative if such colonies are not present or if the identification tests are negative.





10 - READING AND INTERPRETATION

After incubation, the presence of microbial growth is evidenced by the presence of turbidity compared to an un-inoculated control. The characteristic of the growth is closely related to the type or types of cultivated microorganisms.

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, 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.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>C. albicans</i> ATCC 10231	20-25°C / up to 72 h / A	growth
<i>A. brasiliensis</i> ATCC 16404	20-25°C / up to 72 h / A	growth
<i>T. rubrum</i> ATCC 28188	20-25°C / up to 72 h / A	growth

For quality control in the pharmaceutical field refer to the current edition of European Pharmacopoeia.
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 Broth is tested for productivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 20-25°C for up to 72 hours and recording the highest dilution showing growth in Reference Batch (G_{RB}) and in Test Batch (G_{TB}). Productivity is tested with the following target strains: *C. albicans* ATCC 18804, *A. brasiliensis* ATCC 16404, *P. chrysogenum* ATCC 10106, *T. rubrum* ATCC 28188, *M. canis* ATCC 36299, *L. casei* ATCC 393. Productivity is also tested with *C. albicans* ATCC 10231 incubating at 30-35°C for 24 hours according to EP. The productivity index $G_{RB} \cdot G_{TB}$ for each test strain shall be ≤ 1 .

13 - LIMITATIONS OF THE METHOD

- Since Sabouraud Broth is a general-purpose medium with very poor selective properties, bacterial strains will also grow.
- If it is necessary to add antimicrobial compounds to the culture medium, it must be considered that their use may be inhibitory to certain fungi. *Cryptococcus neoformans*, *Trichosporum parapsilosis*, *Candida krusei*, *Candida tropicalis* are sensitive to the combination of cycloheximide, penicillin and streptomycin. Cycloheximide is inhibitory towards saprophytic fungi. Chloramphenicol may be inhibitory towards some pathogenic fungi. Penicillin inhibits *Nocardia* and *Actinomyces*. The combination of cycloheximide and chloramphenicol is inhibitory to the growth of many pathogenic fungi.⁵
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests. If relevant, perform antimicrobial susceptibility testing.
- In clinical microbiology, 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 production process of prepared 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 medium 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.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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 shelf life of the finished products, according to the type and the storage method (temperature and packaging). According to MacFaddin, the tubed medium prepared by the user can be stored at 2-8°C for 6 months.⁵



**16 - REFERENCES**

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacteriologique sur la pluralite des trichophytions de l'homme. Ann Dermatol Syphil 1892; 3:1061-1087.
3. Atlas R, Parks L. Media Handbook of Microbiological Media 2nd ed CRC Press, 1997
4. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> 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	2022/05
Revision 2	Update of Chapters 1, 3, 9, 13,15, 16	2023/03

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

