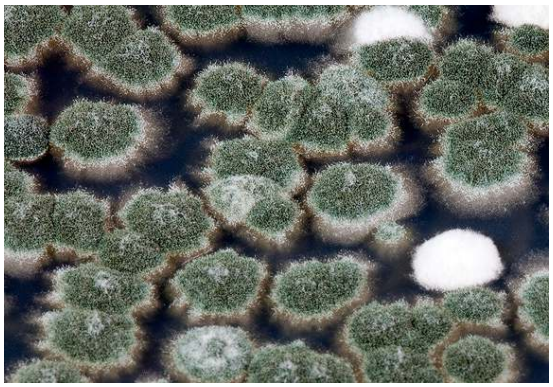
INSTRUCTIONS FOR USE

SABOURAUD DEXTROSE AGAR + CAF

Ready-to-use plates



Aspergillus restrictus
on Sabouraud Dextrose Agar + CAF

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation and enumeration of yeasts and moulds in clinical and non-clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	5.00 g
Peptic digest of meat	5.00 g
Glucose	40.00 g
Agar	15.00 g
Chloramphenicol	0.05 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

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 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 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.
The components of Sabouraud Dextrose Agar conform to the recommendations of the current European Pharmacopoeia[5]. The addition of chloramphenicol is a modification designed to increase bacterial inhibition and improve the isolation of opportunistic fungi from contaminated specimens.
Sabouraud Dextrose Agar + CAF is a selective medium for the isolation of yeasts and moulds, mainly opportunistic pathogens (*Aspergillus, Fusarium, Mucor, Rhizopus*, etc.), cycloheximide sensitive fungi such as *Cryptococcus neoformans*, *Allescheria boydii* and *Candida* spp. in clinical specimens and for the enumeration of yeasts and moulds in non-clinical samples such as cosmetics, as recommended by ISO 16212.[6]
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; the selective properties are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria. Glucose, at high concentration is a carbon and energy source.

4 - PHYSICAL CHARACTERISTICS

Prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.6 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar + CAF	Ready-to-use plates	542006	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 + CAF can be directly inoculated with many clinical specimens collected from various normally non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[7-8] Sabouraud Dextrose Agar + CAF is not suitable for direct inoculation of blood samples or other specimens from normally sterile sites. 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.[7] For cosmetics, refer to the ISO Standard for details of sample collection and preparation.[6]

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.

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Inoculate each specimen in duplicate; incubate one set in aerobic condition at 20-25°C, the other at 33-37°C.[9]
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 enumeration of yeasts and moulds in cosmetics, the technique recommended by ISO 16212[6] and summarized below for surface spread method, should be followed.
Spread over the surface of the medium a measured volume of not less than 0.1 ml of the initial suspension and/or sample dilution.
Incubate at 25°C ± 2.5°C for 3 to 5 days.
The ISO Standard describes also the pour-plate and the membrane filtration methods.

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.
Cosmetics: after incubation, count the colonies in Petri dishes containing 15 to 150 colonies.

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.[10]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C.albicans ATCC 10231	20-25°C / ≤ 5 days/ A	good growth, white yeast-like colonies
T.mentagrophytes ATCC 9533	20-25°C / ≤ 5 days/ A	good growth, white colonies with typical morphology
A.brasiliensis ATCC 16404	20-25°C / ≤ 5 days/ A	good growth, white/black colonies with typical morphology
E.coli ATCC 25922	20-25°C / ≤ 5 days/ A	inhibited

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 + CAF and of the raw material used for the production of prepared plates, dehydrated Sabouraud Dextrose Agar w/CAF 50 (SDA-CAF, Test Batch-TB) are 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; SDA-CAF plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 20-25°C for 3-5 days. 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. *A*fter incubation at 20-25°C for up to 5 days, 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^{-4} of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922, *P.mirabilis* ATCC 10005 and *S.aureus* ATCC 25923. The growth of *E.coli* and *S.aureus* is totally inhibited, the growth of *P.mirabilis* is partially inhibited in both batches.

12 - LIMITATIONS OF THE METHOD

- Chloramphenicol may inhibit pathogenic fungi.[9]
- Sabouraud Dextrose Agar + CAF has a poor efficacy in the isolation of *Histoplasma capsulatum* from potentially contaminated clinical specimens.[11]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of yeasts and moulds with lower selectivity such as Sabouraud Dextrose Agar or Potato Dextrose Agar and with higher selectivity such as Dermatophyte Test medium, should be used.
- 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.

13 - 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.
- This product is not classified as dangerous according to current European legislation.
- 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 the product doesn't contain any transmissible pathogen. 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 by Biolife Italiana 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. Dispose 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.
- 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.

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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TABLE OF APPLICABLE SYMBOLS

REF or 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	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/08
Revision 2	Removal of obsolete classification	2023/03

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

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