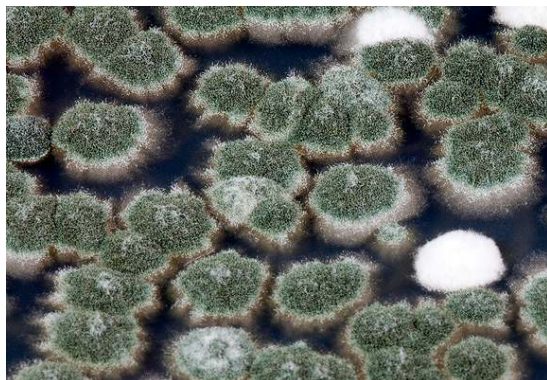


INSTRUCTIONS FOR USE

SABOURAUD DEXTROSE AGAR W/ CAF 50

Ready-to-use flasks

 Sabouraud Dextrose Agar w/CAF 50 : *Aspergillus restrictus*
1 - INTENDED USE

In vitro diagnostic. 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 Spring³, but the suitable medium was selected by Hodges⁴. 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 Pharmacopoeia⁵. 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 w/ CAF 50 mg 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* and *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- METHOD OF PREPARATION

Liquefy the contents of the flask in an autoclave set at $100 \pm 2^\circ\text{C}$ or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to $47-50^\circ\text{C}$ and pour the medium into sterile Petri dishes or tubes, under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

| | |
|---------------------|----------------|
| Medium appearance | yellow, limpid |
| Final pH at 20-25°C | 5.6 ± 0.2 |

6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|----------------------------------|---------------------|---------|---|
| Sabouraud Dextrose Agar w/CAF 50 | Ready-to-use flasks | 5120062 | 6 x 100 mL; 6 glass bottles with flat bottom and aluminium screw-cap; packaging: cardboard box. |
| | | 5120063 | 6 x 200 mL; 6 glass bottles with flat bottom and aluminium screw-cap; packaging: cardboard box. |

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water bath or hot plate, incubator and laboratory equipment as required, sterile plastic Petri dishes, sterile tubes, sterile loops, needles and swabs, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Sabouraud Dextrose Agar w/CAF 50 mg 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 w/CAF 50 mg 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





9 - TEST PROCEDURE

Allow plates and/or tubes to come to room temperature and to dry the surface of the plated medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop 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 medium surface, then streak from this inoculated area. For cutaneous samples, press the 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.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 16212⁶ 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.

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.

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

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.¹⁰

| CONTROL STRAINS | INCUBATION T° / τ / ATM | EXPECTED RESULTS |
|-----------------------------------|-------------------------|---|
| <i>C.albicans</i> ATCC 10231 | 20-25°C / ≤ 5 days/ A | good growth, white yeast-like colonies |
| <i>T.mentagrophytes</i> ATCC 9533 | 20-25°C / ≤ 5 days/ A | good growth, white colonies with typical morphology |
| <i>A.brasiliensis</i> ATCC 16404 | 20-25°C / ≤ 5 days/ A | good growth, white/black colonies with typical morphology |
| <i>E.coli</i> ATCC 25922 | 25-35°C / ≤ 5 days/ A | inhibited |

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 ready-to-use tubes of Sabouraud Dextrose Agar W/CAF 50 and of the raw material used for the production of prepared tubes, dehydrated Sabouraud Dextrose Agar w/CAF 50 (SDA-CAF, 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; SDA-CAF is 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. After incubation at 20-25°C for up to 5 days, the amount of growth 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 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.

13 - LIMITATIONS OF THE METHOD

- Chloramphenicol may inhibit pathogenic fungi.⁹
- Sabouraud Dextrose Agar w/CAF 50 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.

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.
- 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 product 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.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks of Sabouraud Dextrose Agar w/CAF are subject to terminal sterilization by autoclaving.
- 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 flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g. microbial contamination, abnormal turbidity, precipitate, atypical colour).

The user is responsible of the validation of tubes and plates shelf-life, according to the method of storage (temperature and packaging).

16 - REFERENCES

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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 | For single use only | Fragile, handle with care |

REVISION HISTORY

| Version | Description of changes | Date |
|------------|---|---------|
| Revision 1 | Updated layout and content in compliance with IVDR 2017/746 | 2021/09 |
| Revision 2 | Removal of obsolete classification | 2023/04 |

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

