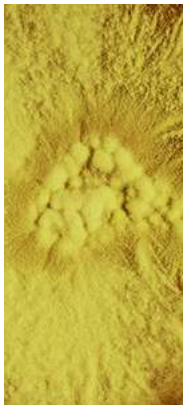
INSTRUCTIONS FOR USE

SABOURAUD DEXTROSE AGAR CAF-CEX

Ready-to-use tubes



Trychophyton mentagrophytes
on Sabouraud Dextrose Agar CAF-CEX

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation and cultivation of dermatophytes and pathogenic yeasts from 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
Cycloheximide	0.5 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] This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories.
The components of the basal medium Sabouraud Dextrose Agar conform to the recommendations of the current European Pharmacopoeia[3]. The addition of chloramphenicol and cycloheximide is a modification designed to increase the selective properties and to improve the isolation of pathogenic fungi, especially dermatophytes, from specimens contaminated with saprophytic fungi and bacteria.
Following the initial report of Whiffen, *et al.*,[4] cycloheximide has been found to be of value in increasing the number of isolations of pathogenic fungi from clinical materials.[5]
Sabouraud Dextrose Agar with CAF-CEX (SDA CAF-CEX) is particularly useful for the investigation of dermatological specimens for superficial mycosis and the target organisms are dermatophytes and some yeasts.[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; glucose, at high concentration is a carbon and energy source. 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 and cycloheximide that inhibits the faster-growing saprophytic fungi.[7]

4 - PHYSICAL CHARACTERISTICS

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

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar with CAF-CEX R	Ready-to-use tubes	552008	20 glass tubes with slanted medium, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

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

7 - SPECIMENS

Tubes of Sabouraud Dextrose Agar with CAF-CEX can be directly inoculated on the slant with clinical specimens collected from sites contaminated with saprophytic fungi and bacteria, mainly skin, nail, hair. Consider that cycloheximide may inhibit some opportunistic fungi (see Limitations of the method). Refer to the quoted literature for specimen types, related to specific infections.[6,8] Sabouraud Dextrose Agar CAF-CEX is not suitable for direct inoculation of 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.[6,8]

8 - TEST PROCEDURE

Allow tubes to come to room temperature.
Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the surface of the medium to obtain well isolated colonies. For cutaneous samples, press specimen lightly into medium.
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 dermatophytes detection incubate at 26-30°C and examine cultures every 4-6 days for a period of up to 21 days.[6]

9 - READING AND INTERPRETATION

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.

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
T.mentagrophytes ATCC 9533	26-28°C / 72 H/ A	good growth, white colonies with typical morphology
S.cerevisiae ATCC 9763	26-28°C / 72 H/ A	inhibited
E.coli ATCC 25922	26-28°C / 72 H/ 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 tubes of Sabouraud Dextrose Agar with CAF CEX is tested for productivity and selectivity.
The productivity characteristics are tested by inoculating the slanted tubes with suitable dilutions of the following target strains: *T.mentagrophytes* ATCC 9533 and *C.albicans* ATCC 10231. *A*fter incubation at 26-28°C for up 72 hours, the amount of growth and colonies' characteristics are evaluated and recorded. The target strains show good growth with white colonies and typical morphology.
The selectivity is evaluated on the slanted tubes with suitable decimal dilutions of the non-target strains *E.coli* ATCC 25922, *A.brasiliensis* ATCC 16404 and *S.cerevisiae* ATCC 9763. The growth of non-target strains is totally inhibited.

12 - LIMITATIONS OF THE METHOD

- Cycloheximide may inhibit some important opportunistic fungi such as *Fusarium*, *Scopulariopsis, Pseudallescheria, Trichosporon*, some *Aspergillus* spp., *Talaromyces* (formerly *Penicillium*) *marneffei*, mucoraceous fungi, some dematiaceous fungi, and yeasts such as *Cryptococcus* spp. and some *Candida* species.[7]
- Some rare non-dermatophyte moulds (*N.dimidiatum, N.hyalinum, Hortaea werneckii*) are capable of causing dermatophyte-like lesions but are inhibited by cycloheximide. If the clinician mentions the possibility of infection with those moulds, the sample should be plated on a cycloheximide-free medium.[6]
- Chloramphenicol may inhibit some pathogenic fungi.[10]
- A single medium is only rarely useful to recover all pathogens contained in a specimen, therefore it is necessary to select media both with and without inhibitory agents for the primary inoculation of the specimen.
- Even if the microbial colonies on the slanted medium 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 these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes 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 tube is for single use only.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass
- Ready-to-use tubes of Sabouraud Dextrose Agar CAF CEX are subject to terminal sterilization by autoclaving.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet 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* diagnostics.
- 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 tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. After opening the box, the tubes can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour).

15 – REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966;9:235-272
2. Sabouraud R. Contribution à l'étude de la trichophytie humaine. Etude clinique, microscopique et bactériologique sur la pluralité des trichophytons de l'homme. Ann Dermatol Syphil 1892; 3:1061-1087.
3. European Pharmacopoeia, current edition.
4. Whiffen AJ, Bonoxos N. Emerson RL. The production of an antifungal antibiotic by Streptomyces qriseus. J Bact 1946; 52: 610-611.
5. Stanley A, Rosenthal D. Furnari BA. The use of cycloheximide-chloramphenicol medium in routine culture of fungi. J Invest Dermatol 1957; 28(5):367-71.
6. Public Health England. Investigation of Dermatological Specimens for Superficial Mycoses. UK Standards for Microbiology Investigations. B 39 Issue 3.1, 2016.
7. Lindsley MD. Reagents, stains and media: mycology. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
8. Berkow EL, McGowan KL. Specimen collection, transport and processing: mycology. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
9. Australian Society for Microbiology: Guidelines for assuring quality of medical microbiological culture media. 2nd Ed, July 2012
10. 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 *In vitro* Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/04
Revision 2	Removal of obsolete classification	2023/04

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

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