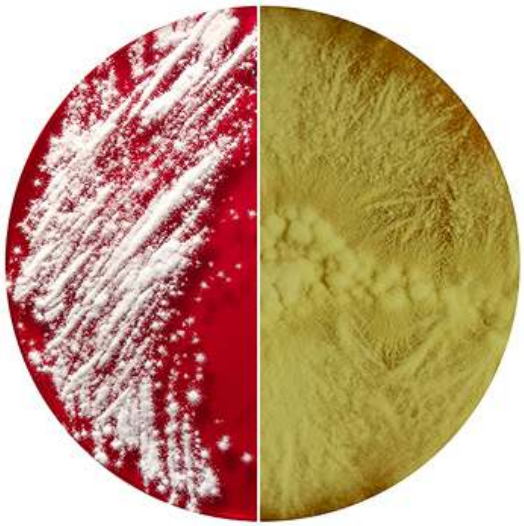
INSTRUCTIONS FOR USE

BIOSECTOR 13
DERMATOPHYTE TEST MEDIUM
SABOURAUD DEXTROSE AGAR + CAF

Ready-to-use bi-plates



Trichophyton mentagrophytes on DTM and SDA +CAF

1 - INTENDED USE

In vitro diagnostic device. Differential and selective media for the detection of dermatophyte fungi from cutaneous specimens such as skin, nails, hair.

2 – COMPOSITIONS- TYPICAL FORMULAS

DEMATOPHYTE TEST MEDIUM *	
Soy peptone	11.0 g
Glucose	10.0 g
Phenol red	0.2 g
Cycloheximide	0.5 g
Gentamicin sulphate	0.1 g
Chlortetracycline HCl	0.1 g
Agar	15.0 g
Purified water	1000 mL
SABOURAUD DEXTROSE AGAR + CAF*	
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

Bi-plates containing Dematophyte Test Medium and Sabouraud Dextrose Agar + CAF can be used for the detection of dermatophyte fungi from cutaneous specimens such as skin, nails, hair.

Dermatophyte Test Medium or DTM has been formulated by Taplin, Zaias and Rebbell[1] in 1969; ready-to-use plates are prepared according to the formula of Taplin *et al.* and are intended for selective isolation and differentiation of dermatophyte fungi responsible for lesions of the skin, nails, hair.[2] Soy peptone provide the nutrients for microbial growth. Glucose is a source of carbon and energy for enhancing dermatophytes growth. Phenol red is a pH indicator, used to detect acid/alkaline production and to differentiate dermatophytes that cultivate with a change to red of the medium because of the production of basic metabolites. The antimicrobials included in the medium partially suppress the growth of bacteria and fungi: cycloheximide inhibits most saprophytic moulds, gentamicin inhibits most Gram-negative and some Gram-positive bacteria, chlortetracycline has a bacteriostatic activity against a wide range of microorganisms including Gram-positive and Gram-negative.

The medium allows the diagnosis of dermatophytes after at least 48 hours of incubation. Allen[3] reported an accuracy of 97% in the identification of dermatophytes with the DTM medium; several authors[4-7] reported that DTM is an effective and convenient medium for confirming dermatophyte infections in Laboratory and in-office.

Sabouraud Dextrose Aga + 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. 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

DEMATOPHYTE TEST MEDIUM	
Prepared plates appearance	orange, limpid
Final pH at 20-25°C	5.5 ± 0.2
SABOURAUD DEXTROSE AGAR + CAF	
Prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.6 ± 0.2

5 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Biosector 13 Dermatophyte Test Medium / Sabouraud Dextrose Agar + CAF	Ready-to-use bi-plates	491013	2 x 10 plates ø 90 mm with 2 sectors primary packaging: 2 cellophane sachets secondary packaging: cardboard box

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

6 - MATERIALS REQUIRED BUT NOT PROVIDED

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

7- SPECIMENS

Biplates with DTM and SDA + CAF are intended for the examination of cutaneous specimens such as nails, hair, skin.[1] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.[2]

8 - TEST PROCEDURE

Allow plates to come to room temperature.
Press cutaneous specimens by gently pressing the samples onto the agar surface of DTM and SDA + CAF.
Incubate aerobically, at 23-27°C for 4-7 days.
Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks.[2]

9 - READING AND INTERPRETATION

After incubation observe the microbial growth and record the specific morphological and chromatic characteristics of the colonies.
On DTM, dermatophytes produce alkaline metabolites which elevate the pH of the medium inducing a colour change of phenol red from orange to red. Examine the medium for evidence of white or light pinkish aerial growth and of a pink to red colour in the medium.
For fast-growing dermatophytes, the red colour appears after 48 hours of incubation; for slow-growing dermatophytes, 3 to 7 days of incubation are required. When there are small colonies, the red colour remains limited to the area around the colony; when the growth is confluent and conspicuous, the indicator changes over the entire plate.
On Sabouraud Dextrose Agar + CAF the same microorganisms grow with the typical chromatic characteristics of the isolated filamentous fungus.

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, it is responsibility of the end-user to perform Quality Control testing 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
DERMATOPHYTE TEST MEDIUM		
T.mentagrophytes ATCC 9533	23-27°C / 94-96h / A	growth, white iphae, the medium turns red-violet
C.albicans ATCC 10231	23-27°C / 94-96h / A	good partially inhibited, white colonies
A.brasiliensis ATCC 16404	23-27°C / 94-96h / A	inhibited
E.coli ATCC 25922	23-27°C / 94-96h / A	inhibited
SABOURAUD DEXTROSE AGAR + CAF		
C.albicans ATCC 10231	23-27°C / 94-96h / A	good growth, white yeast-like colonies
T.mentagrophytes ATCC 9533	23-27°C / 94-96h / A	good growth, white colonies with typical morphology
A.brasiliensis ATCC 16404	23-27°C / 94-96h / A	good growth, white/black colonies with typical morphology
E.coli ATCC 25922	23-27°C / 94-96h / 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 bi-use bi-plates and of the raw materials used for the production of bi-plates, (dehydrated Dermatophyte Selective Medium–DTM- (Taplin), REF 4013691 supplemented with Dermatophyte Antimicrobic Supplement, REF 4240024 and Sabouraud Dextrose Agar w/ CAF 50 mg, REF 402006) are tested for productivity and selectivity by comparing the results with previously approved Reference Batches.

DERMATOPHYTE TEST MEDIUM

Productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *Microsporum canis* ATCC 36229, *Trichophyton rubrum* ATCC 28188, *Trichophyton mentagrophytes* ATCC 9533 After incubation at 23-27°C for 96 hours, typical colonies develop white aerial hyphae with an alkalinisation of the medium that turns to red.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *C.albicans* ATCC 10232, *A.brasiliensis* ATCC 16404, *S.cerevisiae* ATCC 9763, *E.coli* ATCC 25922, *S.aureus* ATCC 25923. *C.albicans* is partially inhibited, the growth of other non-target strains is totally inhibited.

SABOURAUD DEXTROSE AGAR + CAF

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. After 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.

12 - LIMITATIONS OF THE METHOD

- Saprophytic fungi normally do not grow on Dermatophyte Test Medium except when the sample is heavily contaminated; in this case there are later growths, characteristic for the colour of the colonies (black for *Aspergillus niger* and *Cladosporium*, green for *Penicillium* spp.) sometimes with a reddening of the medium: *Candida albicans* cultivates without changing the color of the medium.[8]
- Disregard any colour after 10 days of incubation; it may be due to growth of contaminants.[8]
- A medium containing cycloheximide should not be used when infection with a non-dermatophyte mould is likely or suspected.[2]
- The use of DTM should be combined with morphological study, since dermatophytoides, usually non pathogenic, such as the *Trichophyton terrestre* complex as well as various *Chrysosporium* species and orher nondermathopytic fungi can grow and turn the medium red.[9]
- DTM may uncommonly give false-negative results with some *Microsporum* isolates.[9]
- Chloramphenicol may inhibit pathogenic fungi.[8]
- Sabouraud Dextrose Agar + CAF has a poor efficacy in the isolation of *Histoplasma capsulatum* from potentially contaminated clinical specimens.[10]
- Even if the microbial colonies on the 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 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 biocontamination, 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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10. Unis G, da Silva VB, Severo LC. Disseminated histoplasmosis and AIDS. The role of culture medium for the bronchoscopic clinical specimens Rev Soc Bras Med Trop. 2004;37:234-7.

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 0	First emission in compliance with IVDR 2017/746	2021/09
Instructions for Use (IFU) - Revision 1	Removal of obsolete classification	2023/03

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it