INSTRUCTIONS FOR USE

DERMATOPHYTE SELECTIVE MEDIUM - DTM - (TAPLIN)
DERMATOPHYTE ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplement

Trichophyton mentagrophytes on DTM

1 - INTENDED USE

In vitro diagnostic. Selective and differential medium and selective supplement for the detection of dermatophytes from cutaneous specimens.

2 - COMPOSITION

DERMATOPHYTE SELECTIVE MEDIUM-DTM-(TAPLIN)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Soy peptone	11.0 g
Glucose	10.0 g
Phenol red	0.2 g
Cycloheximide	0.5 g
Gentamicin sulphate	0.1 g
Agar	15.0 g

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

DERMATOPHYTE ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Chlortetracycline HCl	50 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The dermatophyte fungi are classified in three genera: *Epidermophyton* spp., *Microsporum* spp. and *Trichophyton* spp. The most common dermatophyte infections are *tinea pedis* (athlete's foot), *tinea unguium* (nail infection) in adults and *tinea capitis* (scalp ringworm) in children.[1]

Dermatophyte Test Medium or DTM has been formulated by Taplin, Zaias and Rebbell[2] in 1969; the dehydrated medium Dermatophyte Selective Medium and the supplement Dermatophyte Antimicrobic Supplement 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.[1]

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 base and in the supplement 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.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 18.4 g in 500 mL of cold purified water, heat to boiling with agitation and sterilize by autoclaving at 115°C for 10 minutes. Cool to 47-50°C and aseptically add the contents of one vial of Dermatophyte Antimicrobic Supplement (REF 4240024) reconstituted with 5 mL of sterile purified water, under aseptic conditions. Mix well and distribute into sterile Petri dishes or into sterile screw cap bottle/tubes and cool in a slanted position.

5 - PHYSICAL CHARACTERISTICS

Dermatophyte Selective Medium

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates/tubes appearance	orange, limpid
Final pH at 20-25 °C	5.5 ± 0.2

Dermatophyte Antimicrobic Supplement

Freeze-dried supplement appearance	high, soft yellow pastille
Reconstituted supplement appearance	yellow, colourless

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Dermatophyte Selective Medium - DTM- (Taplin)	Dehydrated medium	40136912	500 g (13,6 L)
Dermatophyte Antimicrobic Supplement	Freeze-dried supplement	4240024	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, Erlenmeyer flasks, Petri dishes, tubes, bottles, sterile loops and swabs, ancillary culture media and reagents for the identification of the colonies.

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8 - SPECIMENS

DTM is 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.[1]

9 - TEST PROCEDURE

Allow plates/flasks or tubes to come to room temperature.
Press cutaneous specimens by gently pressing lightly the samples onto the agar surface.
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.[1]

10 - READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
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 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 or flask or tube.

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
T.mentagrophytes ATCC 28185	23-27°C / 94-96h / A	growth, the medium turns red-violet
C.albicans ATCC 18804	23-27°C / 94-96h / A	good partially inhibited
A.brasiliensis ATCC 9642	23-27°C / 94-96h / A	good partially inhibited
E.coli ATCC 25922	23-27°C / 94-96h / 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 dehydrated Dermatophyte Selective Medium–DTM- (Taplin), supplemented with Dermatophyte Antimicrobic Supplement, is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
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 10231, *A.brasiliensis* ATCC 9642, *S.cereviciae* 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.

13 - LIMITATIONS OF THE METHOD

- Saprophytes may redden the medium if specimen material is heavy contaminated but they can be recognized by their dark green or black hyphae; dermatophytes exhibit white aerial hyphae.[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.[1]
- 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.
- The culture medium and the supplement are 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

- The medium base and the supplement are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- 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 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.
- The supplement is sterilised by membrane filtration.
- Many fungi are known to have allergenic effects so care should be taken to limit dissemination of fungal spores.[1]
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.

- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- 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* 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

Dehydrated medium: 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 (plates/tubes/bottles) and the storage method applied (temperature and packaging).

Selective supplement

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

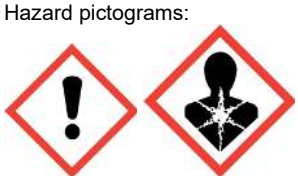
1. Public Health England. Investigation of dermatological specimens for superficial mycoses. SMI B 39, Issue no: 3.1, 2016.
2. Taplin D, Zaias N, Rebbell G, Blank H. Isolation and recognition of dermatophytes on a new medium (DTM) Arch Derm 1969; 99:203-209.
3. Allen AM, Drewry RA, Weaver RE. Evaluation of Two New Color Indicator Media for Diagnosis of Dermatophytosis. Arch Dermatol. 1970;102(1):68–70
4. Elewski BE, Leyden J, Rinaldi MG, Atillasoy E. Office practice-based confirmation of onychomycosis: a US nationwide prospective survey. Arch Intern Med. 2002;162(18):2133-2138.
5. Jennings MB, Rinaldi MG. Confirmation of dermatophytes in nail specimens using in-office dermatophyte test medium cultures. Insights from a multispecialty survey. J Am Podiatr Med Assoc. 2003;93(3):195-202.
6. Rahman MA, Chowdhury OA, Debnath MR, et al. Comparison among Different Culture Media for the Detection of Dermatophytes. Mymensingh Med J. 2018;27(3):626-630.
7. Rich P, Harkless LB, Atillasoy ES. Dermatophyte test medium culture for evaluating toenail infections in patients with diabetes. Diabetes Care. 2003;26(5):1480-1484.
8. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

40136912 - DERMATHOPHYTE SELECTIVE MEDIUM –DTM-(TAPLIN)
SDS rev 5
Regulation (EU) 2020/878

Classification

Germ cell mutagenicity, category 2	H341	Suspected of causing genetic defects.
Reproductive toxicity, category 1A	H360	May damage fertility or the unborn child.
Acute toxicity, category 4	H302	Harmful if swallowed.
Hazardous to the aquatic environment, chronic toxicity, category 3	H412	Harmful to aquatic life with long lasting effects.

Labelling
Hazard pictograms:



Signal words: Warning

Hazard statements:

H341	Suspected of causing genetic defects
H360	May damage fertility or the unborn child.
H302	Harmful if swallowed.
H412	Harmful to aquatic life with long lasting effects.
EUH208	Contains: GENTAMYCIN SULFATE May produce an allergic reaction. Restricted to professional users.

Precautionary statements:

P201	Obtain special instructions before use.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P308+P313	IF exposed or concerned: Get medical advice / attention.
P264	Wash . . . thoroughly after handling.

P273 Avoid release to the environment.
Contains: cycloheximide

4240024 DERMATOPHYTE ANTIMICROBIC SUPPLEMENT
SDS rev 6
Regulation 2020/878

Classification

Reproductive toxicity, category 2	H361fd Suspected of damaging fertility. Suspected of damaging the unborn child.
Eye irritation, category 2	H319 Causes serious eye irritation.
Skin irritation, category 2	H315 Causes skin irritation.
Specific target organ toxicity - single exposure, category 3	H335 May cause respiratory irritation.
Skin sensitization, category 1	H317 May cause an allergic skin reaction.

Labelling
Pictogram

Signal word Warning

Hazard statements:

H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
H335	May cause respiratory irritation.
H317	May cause an allergic skin reaction.

Precautionary statements:

P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P201	Obtain special instructions before use.
P312	Call a POISON CENTRE / doctor / . . . if you feel unwell.
P403+P233	Store in a well-ventilated place. Keep container tightly closed

Contains: chlortetracycline hydrochloride

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/06
Revision 3	Update of “precautions and warnings” and “storage conditions and shelf life”; inclusion of hazard and precautionary statements	2022/04
Revision 4	Removal of obsolete classification	2023/04

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

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