Reading the Results

Evaluation

Microscopically observe the medium for growth and color change without opening the InTray DM by placing the unopened try under a microscope lens to view the organisms using 100x and 200x power. Staining is not required. **See the Identification Chart below**.

Mixed Growth: Dermatophytes and saprophytes (contaminants) will grow on the same tray. The dermatophytes will start to grow first and will turn the media red around the colony. The saprophytes will grow but there will be no color change around the colony until the colony color changes from white to yellow, black, brown, or green.

Positives: If, within 1-14 days, the medium color changes to red at the location of the specimen and whitish colonies grow, the InTray DM is presumptive positive.

Negatives: Trays that show no colony growth or color change 14 days after inoculation are presumptive negative.

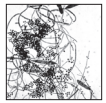
Dermatophyte Identification

The dermatophyte medium changes to red in the presence of these and other common, growing dermatophytes 2 to 14 days after inoculation. The dermatophyte medium changes to red in the presence of these and other common, growing dermatophytes 2 to 14 days after inoculation.

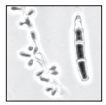
This is a selection of commonly encountered organisms. Please consult our DM Wall Chart (Cat. No. 100-000-005; also available online at biomeddiagnostics.com), for a more detailed selection, and the references listed below, as well as other standard mycology and microbiology references.

Microsporum canis Septate hyphae. Macroconidia: (10-25 x 35-110 µm) numerous, long, spindle-shaped, rough, thick walls are apparent at the knob-like ends. Microconidia: few in number, smooth and club-shaped.

Microsporum gypseum Septate hyphae. Macroconidia: (8-16 x 22-60 µm) large numbers, symmetrical, rough and relatively thin-walled with rounded ends, not pointed like *M. canis*. Microconidia: usually resent, club-shaped.



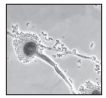
Trichophyton mentagrophytes Septate hyphae. Macroconidia: (4-8 x 20-50 µm) occasionally present, cigar-shaped, thin-walled, narrow attachments to septate hyphae, 1-6 cells, found in young cultures 5-10 days old. Microconidia: usually present in powdery cultures, very round, clustered on branched conidiophores; in fluffy cultures, smaller, fewer, teardrop shaped and easily confused with those of *T. rubrum*.



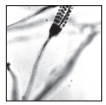
Trichophyton rubrum Septate hyphae. Macroconidia: (4-6 x 15-30 µm) abundant, rare or absent, however can be long, narrow, thin-walled, parallel sides, 2-8 cells, may form on the ends singly or in groups. Microconidia: (2-3 x 3-5 µm) lateral, teardrop shaped, form on macroconidia.

Saprophyte (Contaminants) Identification

***Alternaria* sp.** Hyphae are septate and dark. Conidiophores are septate, variable in length and sometimes branched. Macroconidia are large (7-10 by 23-24 µm), brown, have both transverse and longitudinal stations, and found singly or in chains. They are usually round at the end nearest the conidiophore, producing a club-like shape. Day 10-14: Colony growth with no initial color change. Colony Morphology - Formation of grayish-white, wooly colonies 10 to 14 days after inoculation, that later become greenish black/brown with a light border. It may eventually become covered by short grayish aerial hyphae. Reverse side is black. The medium will change to pink when the colony changes color.



***Aspergillus* sp.** Microscopic morphology – Septate hypae (2.5-8 µm in diameter); unbranched condidiophore arises from a specialized foot cell. The conidiophore is enlarged at the tip, forming a swollen vesicle completely or partially covered with flask-shaped phialides. The pialides produce chains of mostly round, sometimes rough conida (2-5 µm in diameter). Day 10-14: Colony growth with no initial color change. Formation of white, cottony colonies 10 to 14 days after inoculation that later become yellow, green, black or brown. Reverse side is white, goldish, or brown. The medium will change to red when the colony changes color.



***Penicillium* sp.** Microscopic morphology – Septate hyphae (1.5-5 µm in diameter) with branched conidiophores that have secondary branches known at metulae. On the metulae, are flask-shaped phialides that bear unbranched chains of smooth or rough conidia (2.5-5 µm in diameter). The entire structure forms the characteristic "penicillus" or "brush" appearance. Day 10-14: Colony growth with no initial color change. Colony morphology – Surface is at first white, then becoming very powdery, bluish green with a white border. Some less common species differ in color. Reverse is usually white but may be red or brown. DM medium will change to pink/red when the colony changes color.

Limitations

If a fungal infection is strongly suspected and the test result is negative, it may be appropriate to retest giving more care to specimen collection. Media color change and colony growth will vary depending upon patient's physical condition and medical history. Samples from patients who have been treated for ringworm (prescriptions, OTC products herbs, shampoos, etc.) may not grow macroscopic colonies but may still show media color change and microscopic growth with distortions to hyphae and spores.

Some soaps and topical agents may cause an immediate color change. If this occurs, discard the test, wash area and re-sample.
The InTray DM is an agar medium that is susceptible to condensation collection within the inner seal, especially when stored at low temperatures and/or having been exposed to extreme temperature fluctuations. If moisture is visible on the surface of the InTrays, dry them (with the seal removed and InTray label in a position allowing for air flow) under a BSL-2 cabinet just prior to inoculation. There should be no visible droplets of moisture on the surface of the agar when they are inoculated. The surface of the dried medium should be smooth and should not show signs (webbed ribbing pattern on the agar surface) of desiccation.

References

1. Kwon-Chung, K.J. and Bennett, J.E., Medical Mycology, Lea and Febiger: Philadelphia, 1992.
2. Murray, P.R., Baron, ET, Pfaller, M.A., Tenover, F.C., Yolkem, R.H., Manual of Clinical Micro- biology 6th ed., American Society for Microbiology: Washington, D.C. 1995, pp. 709-722.
3. Larone, D.H., Medically Important Fungi: A Guide to Identification, 2nd ed., American Society for Microbiology: Washington, D.C., 1995.

Symbol glossary: biomeddiagnostics.com/l/symbol-glossary

Document Revision History

Rev. K, September 2019
New format; added new catalog numbers, limitation about condensation, document revision history, reference to online symbol glossary; specified 18–25°C instead of room temperature; removed some organisms from organism identification table and added reference to DM Wall Chart and listed references; reorganized and retitled some sections.

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BIOMED

InTray® DM

Enriched Dermatophyte Medium

REF 12-063-001 Σ 5

REF 12-063-002 Σ 20

Not available in all countries; please inquire.

A Selective Culture System for the Identification of Dermatophytic fungi

For *In Vitro* Diagnostic Use

CE IVD 18°C 25°C

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Certificate of Analysis

Introduction

Intended Use

InTray® DM is an enriched dermatophyte medium used in the detection of dermatophytes from clinical specimens.

Description and Principle

Dermatophytes are fungi in the genera *Microsporum, Tricophyton* and *Epidermophyton*. They are capable of metabolizing keratin found in skin, hair and nails of living hosts. The fungi characteristically may invade the cutaneous tissue of the living host but rarely penetrate the subcutaneous tissue. *Tinea* and ringworm are two terms commonly used to describe dermatophytes.

InTray DM is formulated to produce a red color in the presence of growing dermatophytes. Moreover, the medium is formulated to produce distinctive colony growth with typical identifying characteristics both macro and microscopically. The medium inhibits most gram-positive bacteria, gram-negative bacteria, yeast and saprophytic fungi. It is a single exposure system with dynamic built-in components and features that are designed for user compatibility and ease of detection.

Reagents and Appearance

This product contains soytone, carbohydrate, growth stimulants, antimicrobial agents including: cycloheximide, color indicator and agar, with a final pH of 5.6 ± 0.1 at 25°C.

Precautions, Safety and Disposal

For *In Vitro* Diagnostic Use

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

Once the tray has been inoculated and resealed, re-open only in a biological safety cabinet. Because of the potential for containing infectious materials, the tray must be destroyed by autoclaving at 121°C for 20 minutes.

Storage

Upon receipt, store InTray DM at 18-25°C. Avoid refrigeration, freezing or prolonged storage at temperatures greater than 40°C. Do not use an InTray DM if the medium shows signs of deterioration or contamination.

Shelf Life

InTray DM expires 27 months from the date of manufacture.

Procedure

Key notes regarding specimen collection: Specimen collection poses a major uncertainty in using this device.

NAILS - Often collecting viable material from infected nails is difficult because the living organisms are well under the nail itself. For best results, cut nails into small pieces.

HAIR - Samples should be grasped at the uninfected end and several (3-6) small pieces, about 2 cm long, should be cut from the infected portion for inoculation onto the medium.

SKIN - Scrapings should be taken with an inoculation tool that has been moistened with the medium or a sharp blade from the outer ridge of an active lesion. Vesicular fluid is unacceptable for dermatophyte culture. If vesiculated, skin scrapings should be taken from the surface.

Prepare Sample:
Use aseptic technique during specimen collection and handling. Remove any soap residue from the sampling area. Clean the area with 70% alcohol and permit to air dry.

Collect Sample:
InTray DM is designed for culturing hair, skin and nail samples (i.e., cuttings/scrapings). All specimens should be handled according to CDC-NIH recommendations for potentially infectious human serum, blood or other body fluids and materials.

Materials Provided
- InTray DM

Materials Required but Not Provided
- Sterile inoculation tool (cotton swab/forceps)
- Laboratory incubator capable of incubation at 18-25°C in the dark

1 Prepare InTray

Manually pull the lower right corner back, completely exposing the protective seal. Remove the seal aby pulling the tab and discard.

DO NOT REMOVE OR ALTER THE WHITE FILTER STRIP OVER THE VENT HOLE!

2 Inoculate Sample

Inoculate the specimen (hair, nail, or skin scrapings) on the surface of the medium.

Do not let hair overhang the well!

Firmly reseal InTray by pressing the edges of the label and the plastic tray together all around the perimeter. Complete the label with patient information per your laboratory requirements.

Incubation

Incubate inoculated trays for up to 14 days at 18-25°C, in the dark. Observe the trays daily for color changes, through the clear viewing window.

Quality Control

This product has been tested and meets the CLSI (formerly NCCLS) Approved Standard for commercially prepared media (M22-A3). At the time of manufacture, quality control testing is performed on each lot of InTray DM. The ability of the media to support growth and demonstrate expected biochemical reactions and morphology is verified by lot.

Recommended strains for QC testing InTray DM

Test Strain	ATCC®	Expected Result
T. mentagrophytes	**9533**	**Growth**
T. rubrum	**28188**	**Growth**
M. gypseum	**14683**	**Growth**
A. brasiliensis	16404	Significant Inhibition
S. aureaus	25923	Significant Inhibition
E. coli	25922	Significant Inhibition
C. albicans	60193	Significant Inhibition