INTRODUCTION

INTENDED USE

InTray® DM-FungID™ is an enriched dermatophyte medium used to aid in the detection of dermatophyte fungi from clinical specimens with mixed microbiota.

DESCRIPTION & PRINCIPLE OF USE

InTray® DM-FungID™ 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. Dermatophytes are fungi in the genera *Microsporum*, *Trichophyton* 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.

REAGENTS & APPEARANCE

InTray® DM-FungID™ agar appears transparent with a yellow hue & contains soytone, carbohydrates, growth stimulants, antimicrobial agents including: cycloheximide, color indicator and agar in distilled water. Final pH is 5.6 ± 0.1 @ 25°C.

STORAGE & SHELF LIFE

InTray® DM-FungID expires 27 months from the date of manufacture. Upon receipt, store trays at room temperature (18-25C˚). Avoid refrigeration, freezing or prolonged storage at temperatures greater than 40˚C. Do not use trays if the medium shows signs of deterioration or contamination.

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 the infected area is vesiculated, skin scrapings should be taken from the surface.

INSTRUCTIONS FOR USE

SAMPLE COLLECTION

Gather materials needed for the test:

- InTray® DM-FungID™ test(s)
- Disposable gloves and sterile inoculation tool (e.g., cotton swab/forceps/scalpel blade)
- Laboratory incubator

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-FungID™ is designed for culturing hair, skin and nail samples (i.e., cuttings/scrapings). All specimens should be handled according to CDC infectious materials isolation guidelines;
https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html

INOCULATION

Immediately label the tray with patient/sample information and date. Pull back the lower right corner adjacent to the clear window of the tray label until the protective seal is completely visible. Remove the seal by pulling the tab. **Discard** the seal. **DO NOT REMOVE OR ALTER THE WHITE FILTER STRIP OVER THE VENT HOLE!**

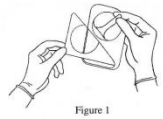


Figure 1

Inoculate the specimen on the **center** surface of the medium. A sterile inoculating loop that has been moistened by touching the surface of the medium may be used for inoculation of solids or scrapings.

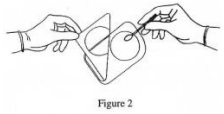


Figure 2

Re-seal all around the tray to ensure a complete seal by pressing the edges of the label against the plastic tray. DO NOT COVER THE VIEWING WINDOW. Complete re-seal prevents dehydration!

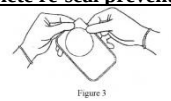


Figure 3

INCUBATION

Incubate inoculated trays in a dark humidified environment for up to 14 days at 18˚C-30˚C. Observe the trays daily through the clear viewing window.

PRECAUTIONS, SAFETY & DISPOSAL

IVD **For professional laboratory use only**. Inoculated trays have the potential of containing live infectious materials, and they must be handled and destroyed in accordance with BSL-2 requirements. Destroy trays by autoclaving at 121°C for 20 minutes or through other suitable means of sterilization.

READING THE RESULTS

MACRO & MICROSCOPIC EVALUATION

Observe the medium for fungal colony growth and red color change. **Without** opening the InTray® DM-FungID™, place the tray-right side up-under a 10x objective microscope lens to view distinct fungal structures (i.e., hyphae, micro/macro-conidia). **See the Quick Identification Chart on the following page for reference**. **Trays to be used with 10x Obj. ONLY!**

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 turns red around whitish fungal colonies, InTray® DM-FungID™ is presumptive positive.

Negatives: Trays that show no colony growth after 14 days from the date of inoculation are presumptive negative.

LIMITATIONS

If a fungal infection is strongly suspected and the culture test result is negative, it may be appropriate to retest giving more care to specimen collection.

- Optimally, simultaneously inoculate samples on both **InTray® SAB-FungID™ w/CC** & **InTray® SAB FungID™** without antimicrobials (e.g., for some pathogenic fungi inhibited by antimicrobials)
- **Transfer of fungal growth to slide examination:** Stained slide preparations can be made as appropriate based on 10x Obj. examination. **InTray® Potato Dextrose Agar (PDA)-FungID™** can be used to induce sporulation.

QUALITY CONTROL

This product has been tested and meets the CLSI Approved Standard for commercially prepared media (M22-A3). At the time of manufacture, quality control testing is performed on each lot of InTray® DM-FungID™. The ability of the media to support growth and morphology is summarized on the Biomed Certificate of Analysis (CoA).

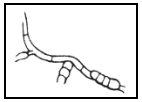
Table 1: Recommended Strains for QC Testing DM-FungID™

Test Strain	ATCC™ Number	Expected Results
T. mentagrophytes	9533	Growth
T. rubrum	28188	Growth
T. tonsurans	28942	Growth
M. gypseum	14683	Growth
A. brasiliensis	16404	Significant Inhibition
S. aureus	25923	Significant Inhibition
E. coli	25922	Significant Inhibition
C. albicans	60193	Significant Inhibition

QUICK IDENTIFICATION CHART (DERMATOPHYTES)

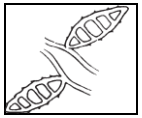
The dermatophyte medium changes to red in the presence of these and other common, growing dermatophytes 2 to 14 days after inoculation

Epidermophyton floccosum



Septate hyphae. Macroconidia: (7-12 x 20-40µm) smooth, thick and thin-walled, club shaped with rounded ends, two to six cells, singly or clusters. Microconidia: none.

Microsporum canis

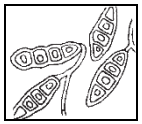


Septate hyphae. Macroconidia: (10-25 x 35-110µm) numerous, long, spindle-shaped, rough, thick walls are apparent at the knob-like ends. Microconidia: few in number, smooth and club-shaped.

Microsporum gallinae

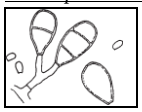
Septate hyphae. Macroconidia are clavate to cigar shaped (6-8 x 15-50µm) and 2 to 10 celled. Microconidia are unicellular and ovoid to pyriform in shape.

Microsporum gypseum



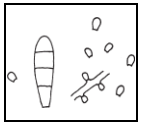
Septate hyphae. Macroconidia: (8-16 x 22-60µm) large numbers, symmetrical, rough and relatively thin-walled with rounded ends, not pointed like *M. canis*. Microconidia: usually resent, club-shaped.

Microsporum nanum



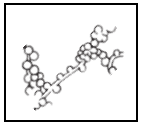
Septate hyphae. Macroconidia (4-8 x 12-18µm) are rough, fairly thin-walled (as in *M. gypseum*), egg-shaped with a truncated base, having usually two cells. Microconidia are club shaped and smooth-walled. Abundance may vary.

Trichophyton equinum



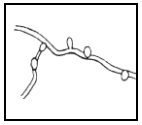
Abundant microconidia which may be clavate to pyriform and sessile or spherical and stalked are formed laterally along the hyphae. Macroconidia are rarely produced, but when present are clavate, smooth, thin walled and of variable size.

Trichophyton mentagrophytes



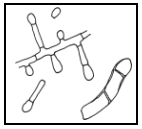
Septate hyphae. Macroconidia: (4-8 x 20-50µm) occasionally present, cigar-shaped, thin-walled, narrow attachments to septate hyphae, 1 - 6 cells, found in young cultures 5 - 10 days old. Microconidia: usually present in powdery cultures, very round, clustered on branched conidiophores; in fluffy cultures,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

Trichophyton rubrum



Septate hyphae. Macroconidia: (4-6 x 15-30µm) 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 3-5µm) lateral, teardrop shaped, form on macroconidia.

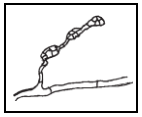
Trichophyton tonsurans



Septate hyphae with variable shaped microconidia all along the hyphae or on short conidiophores that are perpendicular to the parent hyphae. Microconidia are usually teardrop or club-shaped, but may elongate or enlarge to round "balloon" forms. Macroconidia are rare, irregular in form and thick-walled. Many have spiral coils and arthrospores.

QUICK IDENTIFICATION CHART (SAPROPHYTES/ CONTAMINANTS)

Alternaria sp. (Saprophyte)

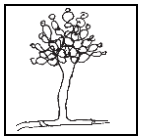


Hyphae are septate and dark. Conidiophores are septate, variable in length and sometimes branched. Conidia are large (7-10 by 23-24µm), brown, have both transverse and longitudinal stations, and found singly or in chains. They are usually round at the end nearest the conidiophore, producing a club-like 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. (Saprophyte)



Microscopic morphology – Septate hypae (2.5 - 8µm 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 phialides produce chains of mostly round, sometimes rough conidia (2-5µm in diameter)

Day 10-14: Colony growth with no initial color change.

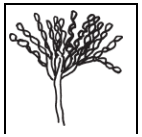
Formation of white, cottony colonies 10 to 14 days after inoculation that later become yellow, green, black or brown. Reverse side is white, goldish, or brown. The medium will change to red when the colony changes color

Candida albicans & Candida tropicalis

Microscopic examination shows clusters of bubbles in the media. Yeast is a common contaminant and there are inhibitors in the media to prevent most yeast species from growing. However, yeast species can be pathogenic depending upon the patient's overall condition and medication history.

Day 1-5: Pink to red color change, no visible growth.

Penicillium sp. (Saprophyte)



Microscopic morphology – Septate hyphae (1.5-5µm 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 (2.5-5µm 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.

Technical notes

In a published study, Singh and Beema found that InTray® DM's, plated as single samples and incubated at 18-25C°, had a dermatophyte organism recovery rate of 85% (99/116) when compared to two other media plated in duplicates (one incubated at 30°C and one at 37°C) as gold standard total (n=116)(6).

In an unpublished study, Davis and Ellis found that InTray® DM showed a 97% dermatophyte recovery rate (70/72) when compared with Mycobiotic agar (also 97%)(7).

REFERENCES

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6) Singh S and Beema PM (2003) Comparative study of different microscopic techniques and culture media for the isolation of dermatophytes, Indian J Med Microbiol 2003;21:21-4. Available from: http://www.ijmm.org/text.asp?2003/21/1/21/8310

7) Davis S and Ellis D (1997) The use of InTray DM agar and DTM agar to isolate and differentiate dermatophytic fungi on colour change alone, Mycology Unit Women's and Children's Hospital, North Adelaide 5006, Australia.

InTray® DM-FungID™

Enriched Dermatophyte Medium

Catalog No. 10-4027 5 Test Kit

Catalog No. 10-4021 20 Test Kit

A SELECTIVE CULTURE SYSTEM TO AID IN THE IDENTIFICATION OF *Dermatophyte fungi*

For *In Vitro* Diagnostic Use

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