

# InTray™ DM

Enriched Dermatophyte Medium

REF Catalog# 10-4007 5 Test Box - 1" well

REF Catalog# 10-4001 20 Test Box - 1" well

For In Vitro Diagnostic Use Only



**BIOMED**  
DIAGNOSTICS

1-800-964-6466 • White City, OR 97503

## INTENDED USE

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

## DESCRIPTION OF THE SYSTEM AND ITS PRINCIPLES

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 used to describe dermatophytoses.

The InTray DM medium is formulated to produce a red color in the presence of growing dermatophytes. When incubated at room temperature, the color change will usually appear within one to 14 days after inoculation. 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.

**SPECIMEN COLLECTION AND HANDLING:** Use sterile technique. Remove any soap residue from the sampling area. Clean the area with 70% alcohol. Air dry. Specimen collections pose the major uncertainties in using the InTray DM. The InTray DM is designed for culturing hair samples, skin scrapings and nail cuttings or scrapings. 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 surface of the medium. Skin scrapings should be taken with 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. Both nail pieces and scrapings from beneath the nail may be cultured. For best results, cut nails into small pieces. See references for specimen collection and handling procedure details. Veterinarians may use "tooth brush" method.

Often collecting viable material from infected nails is difficult because the living organisms are well under the nail itself. If a fungal infection is strongly suspected and the InTray DM is negative, it may be appropriate to retest giving more care to specimen collection.

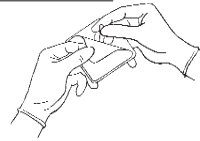
All specimens should be handled according to the CDC-NIH recommendations for potentially infectious human serum, blood or other body fluids and materials. After inoculation, open the InTray DM only in a biological safety cabinet.

## INOCULATION PROCEDURE

Pull back the lower right corner adjacent to the clear window of the InTray DM lid until the protective seal over the agar is completely visible. Remove the seal by pulling the tab and **discard** the seal. (Figure 1)

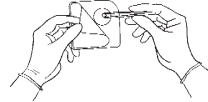
## **DO NOT REMOVE & DO NOT COVER THE WHITE FILTER STRIP OVER THE VENT HOLE!**

Figure 1



Inoculate the specimen (hair, nail or skin scrapings) on the surface of the medium. A sterile inoculating loop that has been moistened by touching the surface of the medium may be used for skin scrapings. Sterile forceps are appropriate for hair or nail cuttings. (Figure 2) **DO NOT LET HAIR OVERHANG THE WELL.**

Figure 2



Reseal the InTray by pressing together the edges of the lid against the plastic tray. **Press all around the InTray to assure a complete seal.** (Figure 3) Label the InTray with patient information and the date. **DO NOT COVER THE VIEWING WINDOW.**

Figure 3



Incubate for as long as 14 days at room temperature (18°-25°C), in the dark. InTrays should be checked daily for color changes. **DO NOT OPEN.** Observe the colony growth and color change through the clear window.

## READING RESULTS

Observe the medium for growth and color change **without opening** the InTray DM.


**Positives for dermatophytes:** If, within one to fourteen days, the medium color changes to red at the location of the specimen and whitish colonies grow, the InTray DM is presumptive positive for dermatophytes.

## Microscopic examination:

Place the entire unopened tray under a microscopic lens to view the organisms using 100x & 200x. Staining is not necessary. Use the Identification Chart attached.

**Negative:** InTrays that show no color change fourteen days after inoculation are presumptive negatives.

## \*\*LIMITATIONS OF THE PROCEDURE\*\*

Growth rates are approximate a  18°-25° (65°-77°).

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.

Soap residue will cause an immediate color change and then the media will revert to the yellow color within 24 hours. These trays are still good to use.


Laboratories licensed for moderately complex tests may identify the organism growing on the medium by microscopic examination through the clear window **without opening** the InTray DM.

## QUALITY CONTROL

This product has been tested and meets the NCCLS Approved Standard for commercially prepared media as required by CLIA '88. At the time of manufacture, quality control testing is performed on each lot of the InTray DM. The ability of the media to support growth and demonstrate expected biochemical reactions and morphology is verified.

Test Strain	ATCC	Expected Results
<i>T. mentagrophytes</i>	9533	Growth
<i>T. rubrum</i>	28188	Growth
<i>T. tonsurans</i>	28942	Growth
<i>M. gypseum</i>	14683	Growth
<i>Aspergillus niger</i>	16404	Significant Inhibition
<i>Staphylococcus aureus</i>	25923	Significant Inhibition
<i>E. Coli</i>	25922	Significant Inhibition
<i>C. Albicans</i>	60193	Significant Inhibition

## STORAGE

Upon receipt, store InTray DM at room temperature,  (18°-25° C). Avoid refrigerating, freezing or prolonged storage at temperatures greater than 40°C. Do not use InTray DM if the medium show signs of deterioration or contamination. Expiration date is 27 months from the date of manufacture.

## REAGENTS











The InTray DM medium contains soytone, carbohydrate, growth stimulants, antimicrobial agents including cycloheximide, color indicator and agar in distilled water.

## PRECAUTIONS

For *in vitro* diagnostic use only. Once the InTray DM has been inoculated and resealed, open only in a biological safety cabinet. Because of the infectious materials involved, the InTray must be destroyed by autoclave or equivalent means of disposal.

## WARNING

This product contains a chemical known to the State of California to cause cancer, birth defects or other reproductive harm.

SYMBOL KEY			
Symbol	Used For	Symbol	Used For
	Batch code		Temperature limitation
	Date of manufacture		Catalog number
	Use by YYY-MM-DD or YYYY-MM		Caution, consult accompanying documents
	Manufacturer		Authorized representative in the European Community
	In vitro diagnostic medical device		in European community

The label on the InTray DM test includes a section where the patient information can be written. The information is: patient #, sample, source, name and date. It is up to the discretion of the practitioner to complete this section or not.

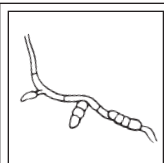
**BioMed Diagnostics, Inc.**

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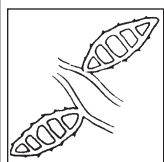
## Dermatophyte Identification Chart



*Epidermophyton floccosum*

Septate hyphae. Macroconidia: (7-12 x 20-40µm) smooth, thin and thick-walled, club shaped with rounded ends, two to six cells, singly or clusters in young cultures; in older cultures macroconidia frequently transform into chlamydoconidia. Microconidia: none.

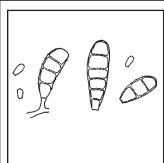
**Day 2-10: Red color change with colony growth**  
*Epidermophyton floccosum* (Dermatophyte)  
Colony morphology - White, cottony



*Microsporium canis*

Septate hyphae. Macroconidia: (10-25 x 35-110µm) numerous, long, spindle-shaped, rough, thick walls are apparent at the knob-like ends, at least 5 compartments. Microconidia: few in number, smooth and club-shaped.

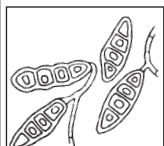
**Day 3-6: Red color change with colony growth**  
*Microsporium canis* (Dermatophyte)  
Colony morphology - White, fluffy to cottony



*Microsporium gallinae*

Microscopic morphology - Produces septate hyphae, macroconidia and microconidia. Macroconidia are clavate to cigar shaped (6-8 by 15-50 µm) and 2 to 10 celled. Most commonly, 5 to 6 cells are observed. They are slightly curved with fine echinulations at their apices. Microconidia are unicellular and ovoid to pyriform in shape.

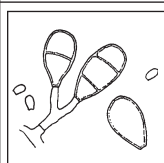
**Day 2-10: Red color change with colony growth**  
*Microsporium gallinae* (Dermatophyte)  
Colony morphology - The surface is slightly fluffy or satiny, white.



*Microsporium 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*, no more than six compartments. Microconidia: usually present, club-shaped.

**Day 2-6: Red color change with colony growth**  
*Microsporium gypseum* (Dermatophyte)  
Colony morphology - White, cottony



*Microsporium nanum*

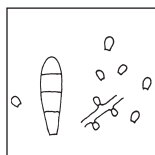
Microscopic morphology - Septate hyphae; macroconidia (4-8 by 12-18µm) are rough, fairly thin walled (as in *M. gypseum*), egg shaped with a truncated base, having usually 2 cells. Microconidia are club shaped and smooth walled and their abundance may vary.

**Day 2-7: Red color change with colony growth**  
*Microsporium nanum* (Dermatophyte)  
Colony morphology - White, cottony

**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.

Most contaminants can be eliminated by: a. using sterile technique and 70% alcohol when collecting the specimen (alcohol kills most contaminants) and b. resealing the InTray so there are no bubbles and gaps around the well.

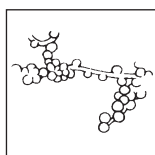
## Dermatophyte Identification Chart



*Trichophyton equinum*

Microscopic morphology - Abundant microconidia which may 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. Occasional nodular organs may be present and the microconidia often undergo a transformation to produce abundant chlamydoconidia in old cultures

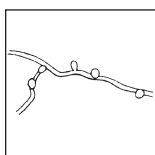
**Day 2-7: Red color change with colony growth**  
*Trichophyton equinum* (Dermatophyte)  
Colony morphology - White cottony



*Trichophyton mentagrophytes*

Septate hyphae. Macroconidia: (4-8 x 20-50µm) occasionally present, cigar-shaped, thin-walled, narrow attachments to septate hyphae, one to six 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 *Trichophyton rubrum*. Spiraled coils and nodular bodies are present in some strains.

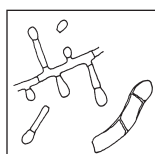
**Day 2-10: Red color change with colony growth**  
*Trichophyton mentagrophytes* (Dermatophyte)  
“athlete’s foot” Colony morphology - White, cottony



*Trichophyton rubrum*

Septate hyphae. Macroconidia: (4-6 x 15-30µm) abundant, rare or absent, however can be long, narrow, thin-walled, parallel sides, two to eight cells, may form on the ends singly or in groups. Microconidia: (2-3 x 3-5µm) lateral, teardropshaped, form on macroconidia. Arthroconidia form on both hyphae and macroconidia. In granular cultures, macroconidia form in larger, rounder numbers than on fluffy strains.

**Day 3-7: Red color change with colony growth**  
*Trichophyton rubrum* (Dermatophyte)  
Colony morphology - White, cottony



*Trichophyton tonsurans*

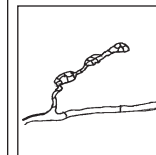
Microscopic morphology - Septate hyphae with many variable shaped microconidia all along the hyphae or on short conidiophores that are perpendicular to the parent hyphae. Microconidia are usually teardrop or club shape, but they may elongate or enlarge to round “balloon” forms. Intercalary and terminal chlamydoconidia are common in older cultures. Macroconidia are rare, irregular in form, and a bit thick walled. Many have spiral coils and arthrospores. Likes to grow into the media not on top.

**Day 3-14: Red color change with colony growth**  
*Trichophyton tonsurans* (Dermatophyte)  
Colony morphology - White at first, may be translucent at first before turning white or orange.

*Color wall chart available from  
Biomed Diagnostics, Inc. Catalog #10-4010*

Also available online at  
[www.biomeddiagnostics.com](http://www.biomeddiagnostics.com)

## Contaminants

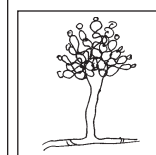


*Alternaria sp.*

Microscopic morphology - 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 septations. Sometimes they produce germ tubes. They are found singly or in chains. They are usually rather round at the end nearest the conidiophore and narrow at the far end producing a clublike shape.

**Day 10-14: Colony growth with no initial color change.**  
*Alternaria sp.* (Saprophyte)

Colony morphology - Surface is at first grayish white and woolly in appearance. Later it becomes greenish black or brown with a light border. It may eventually become covered by short, grayish, aerial hyphae. Reverse side is black. **DM media will change to pink color when colony changes color.**



*Aspergillus sp.*

Microscopic morphology - Septate hyphae (2.5 - 8.0µm in diameter); unbranched conidiophore arises from a specialized foot cell. The conidiophore is enlarged at the tip, forming a swollen vesicle. Vesicles are completely or partially covered with flask-shaped phialides which may develop directly on the vesicle (uniserial form) or be supported by a cell known as a metula (biseriate form). The phialides produce chains of mostly round, sometimes rough conidia (2-5µm in diameter).

**Day 10-14: Colony growth with no initial color change.**  
*Aspergillus sp.* (Saprophyte)

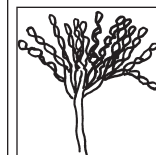
Colony morphology - Surface is at first white, then any shade of yellow, green, black, or brown. Texture velvety or cottony. Reverse is white, gold- ish or brown. **DM media will change to pink or red color when colony changes from white to yellow, green, black or brown.**



*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 and crowding out dermatophyte growth. However, yeast species can be pathogenic depending upon the patient’s over all condition and medication history.

**Day 1-5: Pink to red color change, no visible growth.**  
*Candida albicans* & *Candida tropicalis* (Yeast)



*Penicillium sp.*

Microscopic morphology - Septate hyphae (1.5-5µm in diameter) with branched conidophores that have secondary branches known as metulae. On the metulae, arranged in whorls, 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.**

*Penicillium sp.* (Saprophyte)

Colony morphology - Surface is at first white, then becoming very powdery, bluish green with a white border. Some less common species differ in color and texture. Reverse is usually white, but may be red or brown. **DM media will change to pink or red color when colony changes color.**

### REFERENCES

1. Kwon-Chung, K.J. and Bennett, J.E., *Medical Mycology*. Lea and Febiger: Philadelphia, 1992.
2. Murray, P.R., Baron, ET, Pfäler, M.A., Tenover, F.C., Tenover, R.H., *Manual of Clinical Microbiology* 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.