

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

Materials Provided

- InTray Dermatophyte

Materials Required but Not Provided

- Sterile inoculation tool (e.g., cotton swab/ forceps/ scalpel blade)
- Laboratory incubator capable of incubation at 18-30°C

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 Dermatophyte 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: cdc.gov/infectioncontrol/guidelines/isolation

1 Prepare InTray

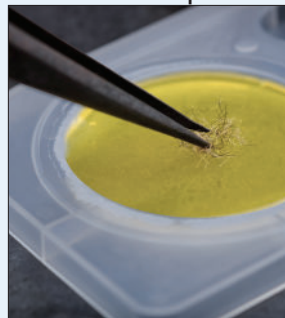


Immediately label the tray with animal/ sample information and date. Pull back the lower right corner adjacent to the clear window of the tray label until the protective seal is completely visible.

Remove the seal by pulling the tab. Discard the seal.

Do not remove or alter the white filter strip over the vent hole!

2 Inoculate Sample



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

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!

Incubation

Incubate inoculated trays for up to 14 days at 18-30°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 the InTray Dermatophyte. The ability of the media to support growth and demonstrate expected biochemical reactions and morphology is verified by lot. Refer to the COA for lot-specific information.

Recommended strains for QC testing of InTray Dermatophyte

Test Strain	ATCC®	Expected Result
<i>T. mentagrophytes</i>	9533	Growth
<i>T. rubrum</i>	28188	Growth
<i>M. gypseum</i>	14683	Growth
<i>A. brasiliensis</i>	16 404	Significant Inhibition
<i>S. aureus</i>	259 23	Significant Inhibition
<i>E. coli</i>	259 22	Significant Inhibition
<i>C. albicans</i>	601 93	Significant Inhibition

Reading the Results

Evaluation

Observe the medium for growth and color change. Without opening the InTray Dermatophyte, place the unopened tray under a microscope lens to view the organisms using the 10x objective (100x power) to view distinct fungal structures (i.e. hyphae, micro/ macro-conidia). Trays to be used with 10x objective ONLY! 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 matures. The colony growth color will change 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 Dermatophyte is presumptive positive.

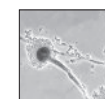
Negatives: Trays that show no colony growth or color change 14 days after inoculation are presumptive negative.

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 hyphae (2.5-8 µm in diameter); unbranched conidiophore 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 (2-5 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

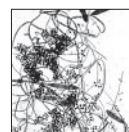


Penicillium sp. Microscopic morphology - Septate hyphae (1.5-5 µm in diameter) with branched conidiophores that have secondary branches known as metulae. On the metulae,

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. Dermatophyte medium will change to pink/ red when the colony changes color.

Dermatophyte Identification

This is a selection of commonly encountered organisms. Please consult our DM Wall Chart (Cat. No. 10-000-004, 10-000-005; 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.

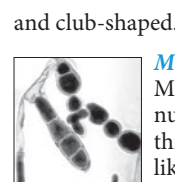


Trichophyton mentagrophytes Septate hyphae. Macroconidia: (4-8 x 20-50 µm) occasionally present, cigar-shaped, thin-walled, narrow attachments to septate hyphae, 1-6 cells, found in young cultures

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*.



Microsporium canis Septate hyphae. Macroconidia: (10-25 x 3.5-110 µm) numerous, long, spindle-shaped, rough, thick walls are apparent at the knob-like ends. Microconidia: few in number, smooth



Microsporium gypseum Septate hyphae. Macroconidia: (8-16 x 2.2-60 µm) large numbers, symmetrical, rough and relatively thin-walled with rounded ends, not pointed like *M. canis*. Microconidia: usually present, club-shaped.