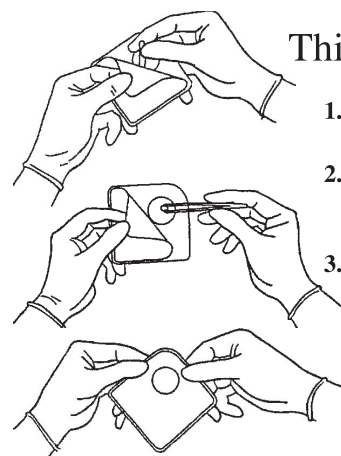


Procedure for Use



This test is very simple to use.

1. Pull back label part way to expose and remove protective inner seal.
2. Inoculate the specially developed medium with hair, nail or skin sample. Veterinarians can use the "toothbrush" method. Do not let hair overhang the well.
3. Reseal label by pressing together the edges of the label against the plastic tray. Press all around the InTray to assure a complete seal. Label the InTray with patient information and the date. **Do not cover the viewing window.**
 - Color will turn in 1 - 3 days.
 - Species specific identification will occur in 3-14 days.
 - The flat design of InTray test slips comfortably on to **your microscope** for easy identification.

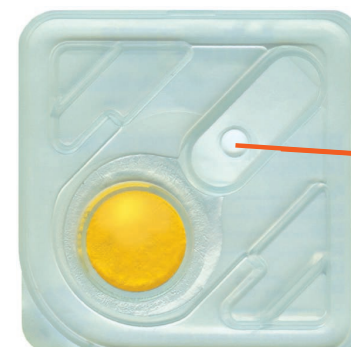
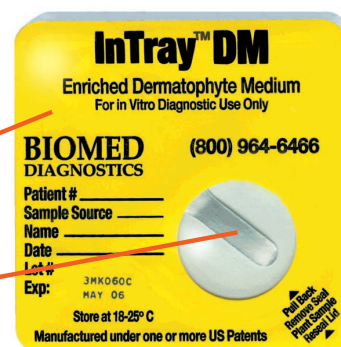
INCUBATION: Incubate inoculated InTrays for up to 14 days at room temperature (18°C - 25°C) in a dark environment. Dermatophytes grow best away from direct light. InTrays should be checked daily for colony growth and for the characteristic red color change. **DO NOT OPEN.** Observe the colony growth and color change through the clear anti-fog window.

InTray™ Dermatophyte Identification Chart

InTray Anatomy:

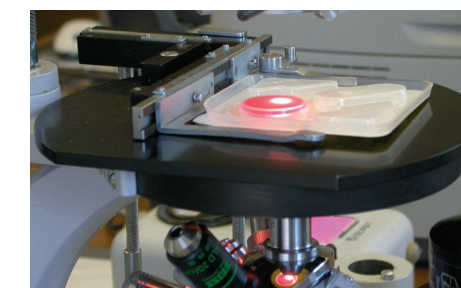
Outer label with clear anti-fog window.

Protective Seal.

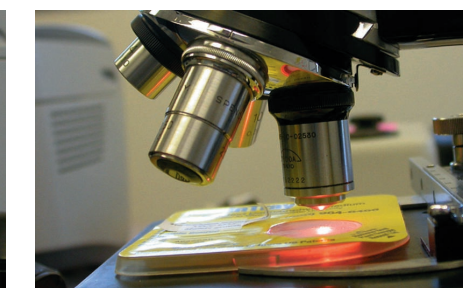


Vent Hole with Tyvek filter.
••• Do not remove filter.
••• Do not cover vent hole.

Microscopic Examination: Staining is not necessary.



Inverted long range microscope:
Place the unopened InTray on the stage with the yellow label facing down.



Compound microscope:
Place the unopened InTray on the stage with the yellow label facing up.

Lens: Start with low power (100x) to determine general growth characteristics. Scan the entire well for at least ten (10) minutes. More than one organism may be observed. Clarification of the micro and macrochondria should use a higher power such as 200x. Be sure to focus up and down through the medium. Some organisms will grow on top of and through the medium.

Microscopic examination should begin as soon as colony growth and/or a color change is noticed. Waiting for an intense red color to develop may make it difficult to illuminate the well. Mature colony growth may be too dense to observe microscopic structures.

DAY 1-5

Candida albicans & Candida tropicalis (Yeast)
Pink to Red Color Change.
No visible growth.

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 2-6

Trichosporum gypseum (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - White, cottony.

Microscopic morphology - Septate hyphae. Macroconidia (8-16 by 22-60 μm) in enormous numbers. Symmetric, rough, relatively thin walled with no more than 6 compartments. The ends are rounded, not pointed as in M. canis. Microconidia are club shaped and are usually present along the hyphae. **Growth can start as soon as 2 days. Matures in 6 days.**

DAY 2

Trichophyton equinum (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - White, cottony.

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. **Matures in 2 - 7 days.**

DAY 2-7

Microsporum nanum (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - White, cottony.

Microscopic morphology - Septate hyphae; macroconidia (4-8 by 12-18 μm) are rough, fairly thin walled (as in M. gypseum), egg shaped with a truncate base, having usually 2 cells. Microconidia are club shaped and smooth walled and their abundance may vary. **Matures in 7 days.**

DAY 2

Epidermophyton floccosum (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - White, cottony.

Microscopic morphology - Septate hyphae; no microconidia. Macroconidia (7-12 by 20-40 μm) are seen best in young cultures. They are smooth, both thin and thicker walled, club shaped with rounder ends. They contain 2 to 6 cells and are found singly or in characteristic clusters. With age, macroconidia often transform into chlamydoconidia. **Matures in 2-10 days.**

DAY 2-10

Trichophyton mentagrophytes (Dermatophyte)"athelete's foot."
Red Color Change with colony growth.
Colony morphology - White, cottony.

Microscopic morphology - Septate hyphae. Macroconidia (4-8 by 20-50 μm) sometimes but not always present. They are cigar shaped, thin walled, have narrow attachment to hyphae, contain 6 cells, and are readily found in young cultures 4-5 days old. The microconidia in powdery cultures are very round and clustered on branched conidiophores. In fluffy colonies they are smaller, fewer, teardrop shaped or "match stick" shaped and can be easily confused with T. rubrum. T. mentagrophytes has more coiled spirals. Nodular bodies are seen in some strains. **Matures in 2-10 days.**

DAY 2

Microsporum gallinae (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - The surface is slightly fluffy or satiny, white.

Microscopic morphology - Produces separate 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. **Matures in 2-10 days.**

DAY 3-6

Microsporum canis (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - White, fluffy to cottony.

Microscopic morphology - Separate hyphae with numerous macroconidia, which are long (10-25 by 35-110 μm), spindle shaped, rough, thick walled, and characteristically taper to knoblike ends. The rough surface of the macroconidia is especially apparent at the knob. Usually more than 6 compartments are seen in the macroconidia. A few microconidia are sometimes observed. They are club shaped, smooth walled, and form along the hyphae. **Growth can start as soon as 3 days. Matures in 6-10 days.**

DAY 3

Trichophyton rubrum (Dermatophyte)
Red Color Change with Colony Growth.
Colony morphology - White, cottony.

Microscopic morphology - Septate hyphae. Macroconidia (4-6 by 15-30 μm) abundant, rare or absent. However they can be long, narrow, thin-walled, parallel sides with 2-8 cells. They may form on the ends singly or in groups. Microconidia (2-3 by 3-5 μm) lateral, teardrop shaped, form on macroconidia. Growth can start as early as 3 days. **Matures at 14 days.**

DAY 3

Trichophyton tonsurans (Dermatophyte)
Red Color Change with colony growth.
Colony morphology - White at first, may be translucent at first before turning white or orange.

Microscopic morphology - Septate hyphae with many variably 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 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. **Matures in 3-14 days.**

Mixed Growth & Artifacts

Mixed Growth Gypseum and Altenaria

Artifact - Hair

Saprophytes are found in the environment.

- On hair shafts and are usually considered to be contaminants
- Can be easily confused with dermatophytes.
- Grow slower on the InTray DM media vs. dermatophytes.
- Identification of these fungi is important in these special conditions when they can be pathogenic.

These special conditions may occur in patients with:

allergies to these fungi	prolonged antibiotic therapy
immunosuppressive drugs	transplants
diabetes	cystic fibrosis
cancer patients	immunologic disorders
equine respiratory diseases	chromomycosis
tuberculosis	

DAY 10-14

Aspergillus sp. (Saprophyte)
Colony Growth with no initial color change.
Colony morphology - Surface is at first white, then any shade of yellow, green, black, or brown. Texture velvety or cottony. Reverse is white, goldish or brown. **DM media will change to pink or red color when colony changes from white to yellow, green, black or brown.**

Microscopic morphology - Septate hyphae (2.5 - 8.0 μm 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 (uniseriate form) or be supported by a cell known as a metula (biseriate form). The phialides produce chains of mostly round, sometimes rough conidia (2.5-5 μm in diameter). **Matures in 10-14 days.**

DAY 10-14

Penicillium sp. (Saprophyte)
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 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.**

Microscopic morphology - Septate hyphae (1.5 - 5 μm in diameter) with branched conidiophores 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. **Matures in 10-14 days.**

DAY 10-14

Alternaria sp. (Saprophyte)
Colony Growth with no initial color change.
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 when colony changes color.**

Microscopic morphology - Hyphae are separate and dark. Conidiophores are septate, variable in length, and sometimes branched. Conidia are large (7-10 by 23-24 μm), 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. **Matures in 10-14 days.**

REFERENCES

1. Kwon-Chung, K.J. and Bennett, J.E., *Medical Mycology*, Lea and Febiger: Philadelphia, 1992.
2. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenoer, 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.

All photomicrographs were taken with a Nikon Coolpix 4500 digital camera attached to a Zeiss Standard UPL (inverted long range) microscope, 100x and 200x phase contrast.

The Zeiss microscope is on loan to BioMed Diagnostics from the Department of Biology at Southern Oregon University, Ashland, Oregon.

The InTray wells were scanned with an Epson Stylus CX5400.

Wall Chart designed and photographed by Joyce Steinbock, MT (ASCP).

Graphics by Roxane Burnett.

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Presumptive Positives:

If, within 1 to 14 days, the InTray medium color changes to red at the location of the specimen and a white, cottony colony grows, the InTray is presumptive positive for dermatophytes. All presumptive positives should be verified by microscopic examination.

Negatives:

InTrays that show no red color change and no white, cottony colony growth after 14 days are presumptive negatives. Yeast and saprophytes may cause a color change with different colony characteristics. Bacteria may be observed microscopically but they are inhibited from growing and they do not cause a color change (Refer to the chart). InTrays showing any red color change should be examined microscopically to identify the organisms.

Additional Dermatophytes:

In research studies the following dermatophyte species have grown on the InTray™ DM medium:

- Trichophyton verrucosum,
- Trichophyton violaceum,
- Trichophyton ajelloi,
- Trichophyton megninii,
- Trichophyton schoenleinii,
- Trichophyton soudanense,
- Microsporum adouinii,
- Microsporum cookei,
- Microsporum vanbreuseghemii.

Uninoculated InTray™ DM medium (agar) should be a clear, yellow color. The well is 3mm deep. Orange, green colors are an indication of contamination and should not be used. Cloudiness and crystals are unacceptable. InTray kits should be stored at room temperature (18°C-25°C) and away from direct sunlight.

Refrigeration will shorten product shelf life and cause deterioration of the anti-fog viewing window.