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## Dynamic Oil Tolnaftate 1%

Lot#: 102015

Antifungal Effectiveness Test

June 29, 2016

Sponsored by MolecularAF

## Protocol for Antifungal Effectiveness

### 1. Test Facility

McClain Laboratories, LLC  
45 Manor Road  
Smithtown, NY 11787

### 2. Test System

*Trichophyton rubrum* (ATCC 28188)  
Microbiologics Quik-stik  
Lot 444-78-7 2017-02 0444 P

MolecularAF  
Dynamic Oil with 1% Tolnaftate (see appendix)

### 3. Test System Identification

Each Petri dish will be labeled with marker or labeled tape.

### 4. Description of Experimental Design

Lyophilized was reconstituted according to manufacturers' specifications. Sabouraud dextrose agar Petri dishes were inoculated with and allowed to incubate at 22c for 5 days until circular growth reached 20mm.

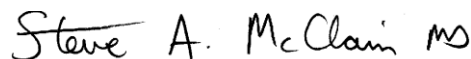
Two filter paper disks containing 1 microliter of Dynamic Oil with 1% Tolnaftate and two disks containing 1 microliter of Bacteriostatic 0.9% Sodium Chloride were placed 2mm from the edge of the colony growth. Incubation was resumed at 22c and observations were made at day 10. The colony and disks were fixed in formalin and processed routinely for microscopic study (H&E and PAS-Alcian Blue).

### 5. Results.

1 microliter of Dynamic Oil with 1% Tolnaftate completely inhibited fungal growth for distances of 2.0mm to 3.2mm from the paper disk and no growth appeared beneath the disks (Fig.A1-3). The disk containing Sodium Chloride demonstrated no inhibition. *Trichophyton rubrum* growing over, around and under the disks (Fig. B1-3), on histopathologic study.

### 6. Observations.

Dynamic Oil with 1% Tolnaftate inhibited *Trichophyton rubrum* over the course of this 5 day study. See attached figures A, B, and appendix.



Steve A. McClain, MD  
Medical Director, McClain Laboratories, LLC



Fig A1

White *Trichophyton rubrum* colony, inhibition by Dynamic oil 1% Tolnaftate. Day 5 on agar before slicing.

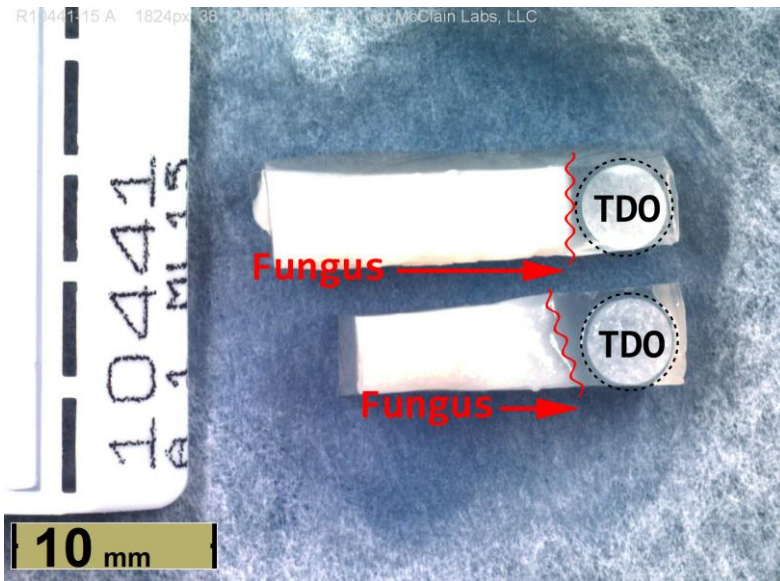


Fig A2

Slices of white *Trichophyton rubrum* colony and round disks with 1% Tolnaftate Dynamic Oil (TDO) after fixation.

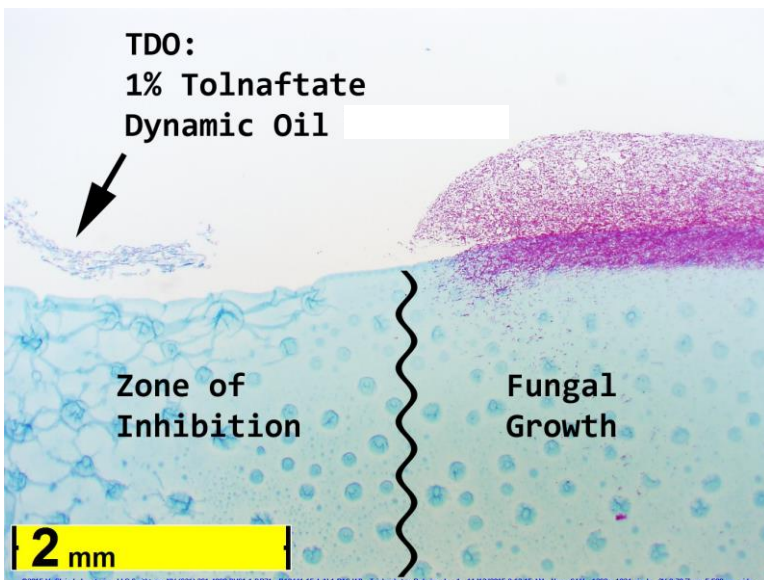


Fig A3- Microscopic

Inhibition of *Trichophyton rubrum*, day 5. Red fungi on blue agar and paper disk with TDO. PAS-Alcian Blue stain, 2x magnification



Fig B1

White *Trichophyton rubrum* colony with saline treated paper disks. No inhibition.

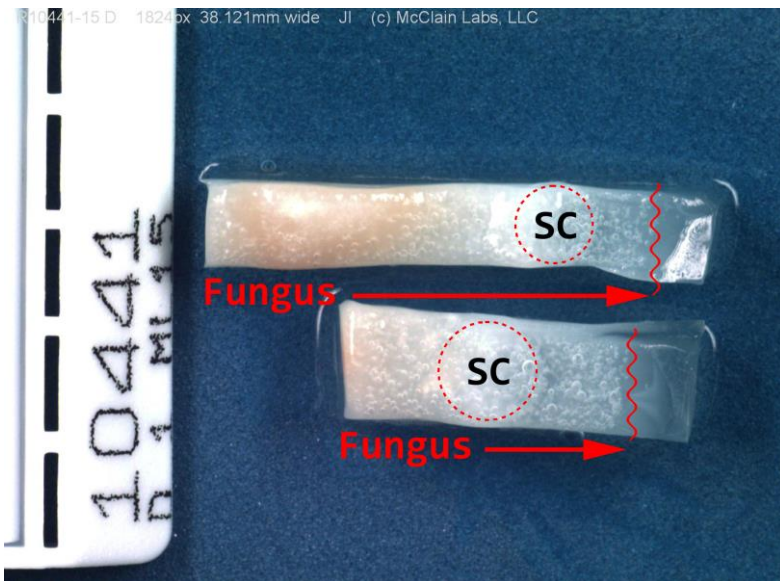


Fig B2

Faint white disks with saline (SC) subsumed by brown-white fungal growth after fixation.

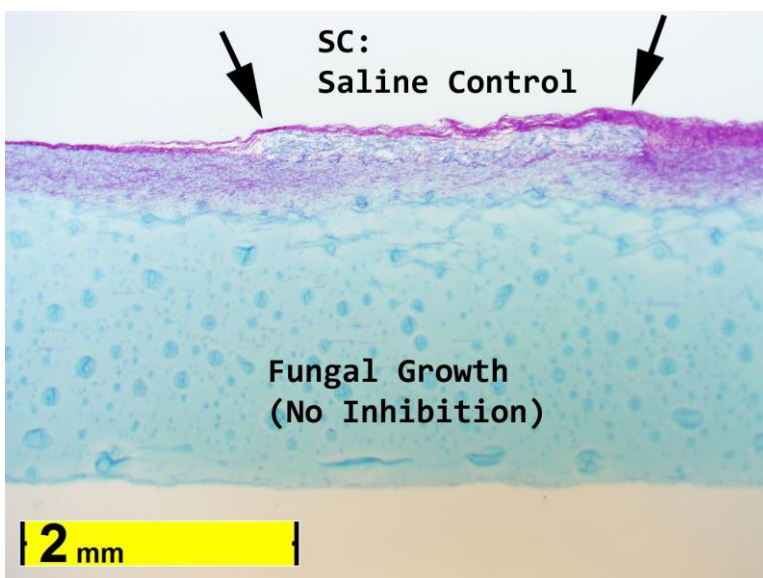


Fig B3-Microscopic

No inhibition of *Trichophyton rubrum*, Day 5. Disk completely overgrown by red fungi on blue agar with normal saline. PAS-Alcian Blue stain, 2x magnification.