PRELIMINARY RESULTS FROM THE EVALUATION OF A SIMPLE DIAGNOSTIC PROCEDURE FOR THE DETECTION OF DERMATOPHYTES IN COMPANION ANIMALS.

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The "InTray" DM (BioMed Diagnostics) is a simple to use medium for the diagnosis of dermatophyte infection. This product was developed for the diagnosis of human dermatophytes and diagnosis is based on colour changes within the media and characteristics of the dermatophyte growth. Preliminary results of an on going study suggest that, based on growth of the dermatophyte and identification of the macroconidia, the test is reliable and accurate. The "InTray" is easy to use and is superior to Wood's lamp which is the common method used in many veterinary practices. Furthermore, "InTray" is as accurate as the standard procedures undertaken at the reference at the Veterinary Faculty, provided that identification of the macroconidia is undertaken.

Objectives of Study

- Evaluate the effectiveness of "InTray".
- Compare "InTray" to other methods; UCD laboratory and Wood's lamp.

Materials and Methods

- Inoculate "InTray" with known dermatophytes.
- Practitioners supplied samples from dermatological cases. "InTray" and standard media in UCD laboratory.
- Questionnaire: Practitioner methods of diagnosis and Wood's lamp.

Results

- "InTray" - Colony Characteristics

Sensitivity	**93%**	**Predictive +ve**	**= 100%**
Specificity	**100%**	**Predictive -ve**	**= 97%**

- "InTray - Colour change

Sensitivity	**93%**	**Predictive +ve**	**= 61%**
Specificity	**80%**	**Predictive -ve**	**= 97%**

- Laboratory Culture

Sensitivity	**87%**	**Predictive +ve**	**= 100%**
Specificity	**100%**	**Predictive -ve**	**= 95%**

- Wood's Lamp (*M. canis* only)

Sensitivity	**60%**	**Predictive +ve**	**= 55%**
Specificity	**85%**	**Predictive -ve**	**= 88%**

Conclusions

Based on preliminary findings:

- **"InTray" is useful providing Colony characteristics are examined**
- **"InTray" is superior to Wood's lamp even if relying on colour change only.**

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Evaluation of a Novel Culture Syustem for the Detection of Dermatophytes. M.L. Elgart and N.G. Warren. The George Washington University Medical Center and the Association of State and Territorial Public Helath Laboratory Directors.

ABSTRACT

Infections due to dermatophytic fungi continue to be a worldwide problem. Definitive diagnosis is obtained when the causative fungus is grown in culture from infected hair, skin, or nails. There are a plethora of media available for use in recovering dermatophytes from clinical samples. Each has its limitations: storage conditions, size and shape or container, test performance, masking of culture color, to name a few. We evaluated a new product (InTray™ DM) with and without an indicator dye for the culture of dermatophytes. This system consists of a 3 cm diameter circle of test medium which, after inoculation, is covered by a clear, permanently attached lid. The entire unit fits on to the stage of a microscope so that a growing culture can be viewed for typical structure without having to make a lactophenol cotton blue teased preparation. A test set of 50 fungi (including all 3 dermatophyte genera and nondermatophytes) were inoculated to the medium and evaluated for growth characteristics and microscopic appearance. Advantages of the product included storage conditions, and ability to make microscopic observations without preparing a smear. Isolates of *Scopulariopsis* sp., *Sporothrix schenckii* and *Phialophora verrucosa* were not typical; *Pseudallescheria boydii* falsely turned the indicator dye positive. This product shows promise as a culturing system for the detection and identification of dermatophytes.

METHODS

Study Design: In order to evaluate the performance of the InTray, a challenge test set of 50 fungi (dermatophytes and nondermatophytes) was inoculated to the system containing media with and without phenol red indicator. Once the appearance of these fungi was determined on the media, then clinical samples, representing specimens obtained from patients suspected of having a dermatophytic infection, were inoculated to the InTray and to routine fungal isolation media.

Challenge Test Set: Fifty fungi were selected to represent dermatophytes and other fungi commonly seen in cutaneous and subcutaneous infections. The fungi were retrieved from the stock culture collection of the Division of Consolidated Laboratory Services (Richmond, Va.) where they had been stored in sterile distilled water at room temperature. The liquid suspension was transferred to Sabouraud dextrose agar and growth was evaluated for typical microscopic and macroscopic morphology. Table 1 lists these fungi.

Clinical Samples: Patients with possible dermatophyte or *Candida* infections were included in the study (see Table 2). Scrapings (skin or hair) were inoculated on standard Sabouraud's dextrose agar and Mycosel agar, as well as on InTray DM (with and without indicator). Media were allowed to remain at room temperature, and were examined daily except weekends. Observations were recorded and lactophenol cotton blue preparations were made of the standard media using clear cellophane tape.

Inoculation of InTray: Following manufacturer's instructions the lid of the tray was pulled back to reveal an aluminum

seal covering the media disk. The aluminum seal was removed completely and discarded. Using a sterile inoculating needle, portions of fungal colony (or clinical sample) were gently touched to the surface of the agar. Excessive pressure was not needed but care was taken to assure that inoculum came in contact with the medium. The tray lid was replaced and the edges were pressed down to achieve a complete seal (see Figures 1-3). The tray was incubated at room temperature and examined daily, except weekends, for color change and/or growth.

DISCUSSION

Dermatophyte infections are among the most common fungal infections seen today in the immune compentent individual. While detection of mycelial elements in a KOH preparation made from skin scales, nail scrapings, or broken hairs offers the presumptive indication that a fungus is present, definitive diagnosis relies on the recovery of the etiologic agent in culture. A number of media have been developed to isolate fungi and all have their obvious merits and/or disadvantages. The one characteristic that all currently recognized dermatophyte media share is the additional need to make a microscopic slide preparation in order view typical fungal structures.

Biomed Diagnostics recently developed a culturing method which simultaneously allows for the culture of clinical material and microscopic viewing of any resultant growth. The design of the packaging permits easy storage of numerous cultured samples. shelf life of the unopened medium is at least six months, and storage and incubation is at room temperature.

This preliminary study was initiated to evaluate the performance of the InTray DM when inoculated with a known set of fungi and with clinical samples from patients suspected of having dermatophytic infections. The culture system was easy to read under the microscope, although early designs of the product caused some blurring at 40X magnification. Noted performance problems (Table 3) observed with the challenge set were of 4 types: atypical colony morphology (4 strains); atypical conidiation (4 strains); growth of a nondermatophyte shifted the pH causing the medium to turn red; and growth of a "saprophytic" fungus on the medium. Because these problems were only seen with the fungi recovered from a culture collection and not from any freshly inoculated clinical specimen, additional challenges are needed to better define the testing limitations. Overall, the InTray was easy to store, use, and interpret.

CONCLUSIONS

- Easy to use in small lab settings
- InTray Media did not dehydrate as quickly as standard plate media
- Good screening technique
- Negative cultures from patients with strong suspicion of fungal infections need further workup
- Needs further evaluation with larger number of isolates (especially the dimorphic fungi)
- Needs further evaluation with larger number of clinical specimens

InTray™DM was provided by Biomed Diagnostics

Table 1 OBSERVATIONS OF SELECTED FUNGI ON IN-TRAY™ DM®

Fungus (No. isolates)	20X microscopic observation	Culture
Microsporum gypseum (5)	ATMm	T
**M. gypseum* (5)	ATMm	P
Microsporum cookei (1)	ATMm	Reverse X
**M. cookei* (1)	ATMm	P
Trichophyton tonsurans (2)	chlamydospores	X
**T. tonsurans* (2)	Tm	P
Microsporum nanum (1)	ATMm	T
**M. nanum* (1)	chlamydospores	P
Trichophyton tonsurans (10)	rare m	T
**T. tonsurans* (10)	rare m	P
Trichophyton rubrum (6)	rare m	T
**T. rubrum* (6)	rare m	P
Trichophyton mentagrophytes (5)	TMm spirals	T
**T. mentagrophytes* (5)	TMm spirals	P
Scopulariopsis sp. (1)	T	T
**Scopulariopsis* sp. (1)	X	NP
Trichophyton terrestre (2)	Tm	T
**T. terrestre* (2)	ATm	P
Sporothrix schenckii (1)	X	X
**S. schenckii* (1)	X	P
Epidermophyton floccosum (2)	chlamydospores, M	T
**E. floccosum* (2)	chlamydospores, M.	P
Scedosporum prolificans (1)	NG	NG
**Pseudallescheria boydii* (1)	T	P
Phialophora verrucosa (1)	X	X
**Alternaria* sp. (1)	TM	T, NP
Trichophyton verrucosum (2)	T	T (growth > 10 days)
Fusarium sp. (1)	X	X restricted growth
Aspergillus fumigatus (1)	NG	NG
Penicillium sp. (1)	NG	NG
Mucor sp. (1)	NG	NG
Microsporum canis (4)	ATMm	T
**M. canis* (4)	ATMm	P

Table 2 OBSERVATIONS OF CLINICAL SPECIMENS ON INTRAY™ DM[a]

Specimen source/ presentation	Organism isolated	Growth on standard media[b]	Growth on InTray
Broken hairs	*T. tonsurans*	AT	ATP
Alopecia	*T. tonsurans*	AT	ATP
Alopecia	*Rhodotorula* sp	AT (SDA only)	NG
Onycholysis	*T. rubrum*	AT	ATP
Onycholysis	*Aspergillus* sp.	AT	NG, NP
Onycholysis	*T. mentagrophytes*	AT	ATP
Onycholysis	*Penicillium* sp.	AT	NG, NP
Onycholysis	*T. rubrum* and *Scopulariopsis* sp.	AT (yellow) AT (SDA only)	ATP NG, NP
Toe web maceration	*T. mentagrophytes*	AT	AT
Chronic scale, foot and hand	*T. rubrum*	AT	ATP
Tinea corporis	*T. mentagrophytes*	AT	ATP
Left hand	*T. rubrum*	AT	ATP
Buttock	*T. rubrum*	AT	ATP
Fingernail	*Penicillium* sp.	AT (SDA only)	NG

[a]abbreviations used:
A = abundant , P = indicator turned red, NP = indicator did not turn red, NG = no growth, , SDA = Sabouraud dextrose agar,
T = typical, X = not typical, M = macroconidia present, m = microconidia present

Table 3 FUNGI EXHIBITING ABERRANT GROWTH ON INTRAY™DM

Fungus	Problem
Microsporum cookei	reverse of the culture was not typical--too pale
Trichophyton tonsurans	2 of 12 strains did not have typical conidiation and the colony texture was too fluffy
Scopulariopsis sp	atypical conidiation
Sporothrix schenckii	atypical conidiation, colony texture was too fluffy; turned indicator red after prolonged incubation (12 days)
Pseudallescheria boydii	turned indicator red
Fusarium sp	growth