Dermatophyte Isolation Media: Quantitative Appraisal Using Skin Scales Infected with *Trichophyton* *mentagrophytes* and *Trichophyton rubrum*

JAMES T. SINISKI,* LEE M. KELLEY, PATRICIA M. FLYNT, AND JOANNE MIEGEL

Department of Microbiology and Medical Technology, University of Arizona, Tucson, Arizona 85721

Received for publication 30 August 1976

Various dermatophyte isolation media were quantitatively appraised by colony counts for their ability to support growth when suspensions of trypsin-treated infected-skin scales were used as inocula. Evaluation included guinea pig skin scales infected with a granular *Trichophyton mentagrophytes*, and one trial was conducted using human skin scales infected with *Trichophyton rubrum*. No differences in the ability to support growth were observed among Taplin Dermatophyte Test Medium, Dermatophyte Test Medium (Schering Diagnostics, Port Reading, N.J.), Sabouraud medium plus cycloheximide and chloramphenicol, Youssef medium for dermatophytes product no. 103^S semisynthetic lots no. 106 and no. 107. With skin scales not grossly contaminated with bacteria, similar counts were obtained with Littman oxgall agar without antibiotics. Youssef medium for dermatophytes product no. 103^N natural lot no. 109 was significantly different from the other media in that lower counts were obtained.

An evaluation of Taplin Dermatophyte Test Medium (T-DTM; Difco) Pfizer DTM, Sabouraud medium plus cycloheximide and chloramphenicol (SAB), and Littman oxgall agar without antibiotics (LITT) has been reported using quantitated spore preparations of *Trichophyton mentagrophytes* (6). Since that evaluation, a technique to obtain homogeneous suspensions of fungal particles in scrapings from infected skin was devised that permits delivering more uniform inocula to culture media (7). Quantitative appraisal of dermatophyte isolation media using fungal particles obtained from scrapings from infected skin would more closely approximate the conditions under which the media would be used in the laboratory for the isolation and growth of dermatophytes. This is a report of the appraisal of various dermatophyte isolation media using such homogeneous suspensions of fungal particles.

MATERIALS AND METHODS

Preparation of skin scales infected with *T. mentagrophytes*. *T. mentagrophytes* var. (*granulare*) *mentagrophytes*, culture B 1343, supplied by L. K. Georg from Center for Disease Control, Atlanta, Ga., was used to infect guinea pigs for the first evaluation. For the second evaluation, *T. mentagrophytes* var. (*granulare*) *mentagrophytes*, culture number 3525 from our mycology laboratory was used. All infections were induced as previously described (7). Ten days after the animals were inocu-

lated a large amount of skin scales and hair was collected. These collections were stored in large, 6-inch (ca. 15.3 cm), glass petri dishes.

Acquisition of skin scales infected with *Trichophyton rubrum*. The dermatologists of Tucson were asked to screen their patients with the objective of obtaining a weighable quantity of skin scales infected with a dermatophyte. One such sample was obtained from a patient with suspected *T. rubrum* infection of the buttocks. The sample was immediately transported after acquisition from the clinic to the laboratory and processed 1 week after it was obtained.

Digestion of infected-skin materials. Skin and hair from infected guinea pigs were mixed in petri dishes to get as homogeneous a collection as possible. A sample weighing 0.005 g was used for a preliminary assay. One milliliter of a 0.5% (1:300) trypsin solution and 1 ml of 0.85% saline solution containing 0.02% Tween 80 were used to digest the skin scales for 1 h as previously described (7). The number of viable particles obtained after 1 h of digestion was ascertained using SAB before the evaluations were performed.

The human skin scale sample weighed 0.0075 g. No preliminary counts were made of this sample. The entire sample was digested using 1.5 ml of a 0.5% (1:300) trypsin solution plus 1.5 ml of saline-Tween 80 solution.

Media evaluated. Two separate batches of DTM in short glass tubes were supplied by Schering Diagnostics (Port Reading, N.J.) and stored in a refrigerator at 4°C after receipt. (This medium is no longer available.) One batch of tubes, all of the same lot number, was supplied several months before so that

the expiration date supplied by the manufacturer was 1 month past due at the time of the first evaluation (S-O-DTM). A second batch, all of another lot number, with the expiration date not past was also used (S-N-DTM). Three other media were prepared in this laboratory and poured into glass petri dishes so that all the media used in the first evaluation were in glass: SAB (3); LITT (Difco Laboratories, Detroit, Mich.); T-DTM (8). Each was prepared in one-batch lots following the essential steps given by Barry and Fay (1) to produce agar media for optimal results. After the SAB was autoclaved, it was cooled to approximately 50°C before adding the antimicrobials: cycloheximide (Actidione, The Upjohn Co., Kalamazoo, Mich.) in acetone and chloramphenicol (Chloromycetin, Parke-Davis and Co., Detroit, Mich.) in 95% ethyl alcohol. Twenty milliliters of each medium was pipetted into separate glass petri dishes (100 by 15 mm).

For the second evaluation, Youssef medium for dermatophytes was supplied by Mycogel Laboratory, Inc., Brooklyn, N.Y. This medium was in plastic petri dishes and was refrigerated upon receipt. Three separate media were supplied: Youssef medium for dermatophytes product no. 103^s, semisynthetic lot no. 106 (no. 106); Youssef medium for dermatophytes product no. 103^s, semisynthetic lot no. 107 (no. 107); Youssef medium for dermatophytes no. 103ⁿ, natural lot no. 109 (no. 109). SAB, LITT, and T-DTM were also prepared as already described for the first evaluation, but 20 ml of these media was pipetted into plastic petri dishes.

For the third evaluation with infected-human-skin scales, media from the second evaluation were used.

Inoculation of media. Based on the original particle count secured after the digestion of portions of skin scales infected with *T. mentagrophytes* for the first and the second evaluations, dilutions were made so that three to four colonies would be expected on each medium for the first evaluation. This low colony count was considered reasonable for the limited surface area of the S-O-DTM and S-N-DTM supplied in glass tubes. Dilutions were made for the second evaluation so that 10 to 20 colonies would be expected on each medium, all of which were in uniformly sized plastic petri dishes. For the third evaluation, other than the fluid used to digest the human skin scales, no further dilutions were made of this material, and it was inoculated onto the various media in that concentration. To insure the random inoculation of the media, selection of the order of placement was determined by chance before each trial. For the first evaluation 0.05 ml was used. For the second and third evaluations 0.1 ml was used per plate. Because of incomplete digestion, a few skin scales could be seen in the fluid as they floated to the top of the pipette. For this reason the last 0.2 ml in the pipette was never used. The fluid was distributed with glass spreaders to the surface of the medium. For the first evaluation, the fluid was distributed to an area equal to that of the agar surface in the tubed media. A template of the area was placed below each plate to indicate the surface area to be spread. For the second and third evalua-

tion, the fluid was distributed over the entire surface of the agar in the petri dish because all media were poured into similar containers. The inoculum on LITT dispersed itself over the surface of the plates and was not mechanically distributed. After spreading the inocula onto the S-O-DTM and S-N-DTM in the first evaluation, the tubes were placed in a holder that kept the agar surface parallel to the horizontal plane. The tubes were removed from the holder after 24 h.

The first evaluation consisted of five separate trials performed on 5 consecutive days with three containers of each medium per trial. For the second evaluation, six separate trials using three petri dishes of each medium were performed on six separate days. The third evaluation involved only one trial using three separate petri dishes of each medium. Colony counts for the first and second evaluation were obtained 10 days after inoculation and were rechecked at 20 days before the plates or tubes were discarded. For the third evaluation, the colony count was made 4 days after inoculation when the colonies were just visible. This count was checked for accuracy for 4 more days. The colony counts, $\times$, were transformed ($\sqrt{(\times) + 0.5}$) for analysis of variance.

RESULTS

First evaluation. In the first evaluation there were no significant differences in counts obtained among S-O-DTM, S-N-DTM, T-DTM, SAB, and LITT. The analysis of variance (Anova) was performed according to a randomized block design with subsamples. Colony counts on all media varied from zero to five. The analysis is summarized in Table 1. Since the ratio of mean squares of media to sample error is not statistically significant, the null hypothesis is accepted; that is, there are no significant differences among the counts on the five media. Because the null hypothesis could not be rejected, the probability of accepting a false hypothesis (type II error) was estimated using the mean plate counts. The largest difference in means of colony counts among media was between SAB and S-N-DTM (0.202). Thus, the probability of a type II error or accepting a false hypothesis (where probability of type I error, α , equaled 0.05) was 0.10. Table 2 lists the means of the colony counts.

Second evaluation. In the second evaluation there was a significant difference between no. 109 and the other media, SAB, T-DTM, LITT, no. 106, and no. 107. Anova was performed according to a randomized block design with subsamples. Colony counts on medium no. 109 were all negative. Colony counts on the other media ranged from 1 to 28. The Anova for all media in the second evaluation is summarized in Table 3. There are significant differences implied in trials and media. Because of the

TABLE 1. Summary of analysis of variance of colony counts of *T. mentagrophytes* grown on S-O-DTM, S-N-DTM, SAB, LITT, and T-DTM^a after plating with a quantitated fungal particle suspension from trypsin-treated skin scales

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	149	10.2153		
Trials (blocks)	4	4.6603	1.1651	8.50
Media	4	1.1306	0.2827	2.07 ^b
Sample error	16	2.1920	0.1370	
Subsample error	125	2.2324	0.0179	

^a S-O-DTM, Schering old Dermatophyte Test Medium; S-N-DTM, Schering new Dermatophyte Test Medium; SAB, Sabouraud medium with antimicrobials; LITT, Littman oxgall agar; T-DTM, Taplin Dermatophyte Test Medium.

^b Not significant.

TABLE 2. Means of colony counts (transformed data) secured when *T. mentagrophytes* was grown on various media after plating with a quantitated fungal particle suspension from trypsin-treated skin scales

Medium ^a	Means of colony counts
S-O-DTM	1.2400
S-N-DTM	1.2672
SAB	1.0652
LITT	1.3041
T-DTM	1.1482

^a See Table 1, footnote *a*.

zero counts on medium no. 109, a second analysis of variance (Anova) was performed, omitting data from medium no. 109. The Anova for the remaining media in the second evaluation is summarized in Table 4. Medium no. 109 is different from the other media, but no significant differences were observed among the remaining media.

In the same manner as with the first evaluation, the probability of a type II error was estimated using the mean plate counts. The largest difference (2.06) in means of colony counts among those media (with omission of no. 109) was between T-DTM and no. 107. The probability of a type II error or accepting a false hypothesis (where $\alpha = 0.05$) was 0.10. Table 5 lists the means of the colony counts.

Third evaluation. In the third evaluation there were differences between media no. 109 and LITT when compared to SAB, T-DTM, no. 106, and no. 107. The Anova was performed according to a one-way analysis. A colony count of one was obtained on two of the three petri dishes of medium no. 109 with the third having no growth. There was gross bacterial

contamination with the colonies of the fungal pathogen on LITT without antimicrobials. Because an accurate colony count of the fungus could not be obtained, this medium was dropped from the analysis. Colony counts for the remaining four media varied from 45 to 98.

TABLE 3. Summary of analysis of variance of colony counts of *T. mentagrophytes* grown on T-DTM, LITT, SAB, Youssef no. 109, Youssef no. 107, and Youssef no. 106^a after plating with a quantitated fungal particle suspension from trypsin-treated skin scrapings

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	107	157.1370		
Samples	35	137.7677		
Runs	5	45.7589	9.1517	12.252 ^b
Media	5	73.3352	14.6670	19.636 ^b
Sample error	25	18.6735	0.7469	
Subsample error	72	19.3642	0.2689	

^a T-DTM, Taplin Dermatophyte Test Medium; LITT, Littman oxgall agar; SAB, Sabouraud medium plus antimicrobials; Youssef no. 109, Youssef medium for dermatophytes no. 103^b, lot no. 109; Youssef no. 107, Youssef medium for dermatophytes no. 103^b, lot no. 107; Youssef no. 106, Youssef medium for dermatophytes no. 103^b, lot no. 106.

^b Significant at 95% confidence limit.

TABLE 4. Summary of analysis of variance of colony counts of *T. mentagrophytes* grown on T-DTM, LITT, SAB, Youssef no. 107, and Youssef no. 106^a after plating with a quantitated fungal particle suspension from trypsin-treated skin scrapings

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	89	86.9991		
Samples	29	67.6348		
Runs	5	54.9116	10.9823	23.070
Media	4	3.2024	0.8006	1.682 ^a
Sample error	20	9.5208	0.4760	
Subsample error	60	19.3642	0.3227	

^a See Table 3, footnote *a*.

^b Not significant.

TABLE 5. Means of colony counts (transformed data) secured when *T. mentagrophytes* was grown on various media after plating with a quantitated fungal particle suspension from trypsin-treated skin scales

Medium ^a	Means of colony counts
T-DTM	9.28
LITT	10.00
SAB	9.39
Youssef no. 107	7.22
Youssef no. 106	7.61

^a See Table 3, footnote *a*.

The Anova for the third evaluation including the results on no. 109 is given in Table 6. Medium no. 109 is different for the other media evaluated but no difference exists among the others remaining. In the same manner as with the first two evaluations the probability of a type II error was estimated by using the mean plate counts. The largest difference (1.576) in means of colony counts among these four media was between no. 106 and no. 107. The probability of a type II error (where $\alpha = 0.05$) was 0.10. Table 7 lists the means of the colony counts for this evaluation. Because medium no. 109 did not support growth of the hyphae from the digested skin scales, an inoculation of spores and mycelium from a mature colony of *T. mentagrophytes* grown on SAB was made to medium no. 109. Lot no. 109 was found to support growth of this organism after such a transfer.

DISCUSSION

In other clinical studies used to determine the efficiency of various dermatophyte isolation media, differing results have been obtained. A review of these studies before 1972 has been made (6). Another study using clinical material from 90 specimens isolated five different taxa of dermatophytes on Mycosel (BBL) and

TABLE 6. Summary of analysis of variance of colony counts of *T. rubrum* grown on T-DTM, LITT, SAB, Youssef no. 109, Youssef no. 107, and Youssef no. 106 after plating with a quantitated fungal particle suspension from trypsin-treated human skin scales

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	14	139.85336		
Media	4	134.45016	33.61254	62.21 ^b
Error	10	5.40320	0.54032	

^a See Table 3, footnote a.

^b Significant difference.

TABLE 7. Means of colony counts (transformed data) observed when *T. rubrum* was grown on various media after plating with a quantitated fungal particle suspension from trypsin-treated human skin scales

Medium ^a	Means of colony counts
T-DTM	8.545
SAB	7.998
Youssef no. 107	9.343
Youssef no. 106	7.767
Youssef no. 109	1.052

^a See Table 3, footnote a.

Sabouraud medium plus gentamicin (5). Seven positive isolations were made using the Mycosel and eight positive cultures were made using Sabouraud medium plus gentamicin. The conclusion of the authors (5) that the results obtained in all these clinical studies varied because of factors that cannot be quantitated appears valid. These approaches also do not lend themselves to statistical analysis.

Our efforts to quantitate the efficiency of dermatophyte media first utilized homogeneous spore suspensions. This was followed by the development of the trypsin digestion for obtaining homogeneous suspensions of fungal material from infected-skin scales. It is believed that this digestion approach utilizes the fungus in the same form as would be found in a specimen directly obtained from an infected human or other animal. The results of using fungal particles derived from trypsin digestion of infected-skin scales to evaluate the efficiency of certain dermatological media led to the same conclusion as when quantitative spore suspensions were used.

When Youssef medium for dermatophytes product no. 103^s was evaluated with SAB, DTM, and LITT, two lots of the Youssef medium were found to be similar to the other media in their ability to support growth; these were lots no. 106 and no. 107. Lot no. 109 did not support the growth of colonies from trypsin-digested guinea pig scales with *T. mentagrophytes* but supported the growth of this species when spores and hyphae from a mature culture were inoculated directly onto it. Lot no. 109 did support very minimal growth of *T. rubrum* in human skin scales after trypsin treatment. The Youssef media for dermatophytes are patented or have pending patent application. The media all contain dextrose, peptone, cycloheximide, and chloramphenicol. Lot no. 106 also contains anthraquinone and sodium citrate; lot no. 107 contains anthraquinone and yeast extract; and lot no. 109 contains Cassia neutral extract and yeast extract.

It is not known why no. 109 did not support the growth of the digested hyphal elements of *T. mentagrophytes* but did support the growth of spores and hyphae from mature colonies when directly placed onto this medium. No growth was obtained on no. 109 when particle counts of 1 to 28 of *T. mentagrophytes* were used, but 1 colony per dish in 2 out of 3 dishes was obtained on no. 109 when particle counts of 45 to 98 of *T. rubrum* were used. It is speculated that adaptive enzymes may be the cause of this phenomenon.

The incorporation into mycological media of

gentamicin as a substitute for other antibacterial antibiotics may be the best choice at this time. The evaluations made up to the present time but without analysis of quantitative inoculations indicate gentamicin to be an effective substitute (2, 5).

From the data secured in the third evaluation, it is now known that trypsin digestion works equally well on human skin scales as compared to guinea pig scales. Parallel results were obtained when the causal organism was either *T. rubrum* or *T. mentagrophytes*.

ACKNOWLEDGMENTS

This work was funded by grants from the Schering Corp., Bloomfield, N.Y. and Mycogel Laboratory, Inc., Brooklyn, N.Y.

LITERATURE CITED

1. Barry, A. L., and G. D. Fay. 1972. A review of some common sources of error in the preparation of agar media. *Am. J. Med. Technol.* 38:241-245.
2. Dolan, C. T. 1971. Optimal combination and concentration of antibiotics in media for isolation of pathogenic fungi and *Nocardia asteroides*. *Appl. Microbiol.* 21:195-197.
3. Emmons, C., C. Binford, and J. Utz. 1970. *Medical mycology*. Lea and Febiger, Philadelphia.
4. Jones, H. E., J. H. Reinhardt, and M. E. Rinaldi. 1974. Acquired immunity to dermatophytes. *Arch. Dermatol.* 109:840-848.
5. Merz, W. G., G. Sandford, and G. L. Evans. 1976. Clinical evaluation of the addition of gentamicin to commercially prepared mycological media. *J. Clin. Microbiol.* 3:496-500.
6. Sinski, J. T., J. R. Swanson, and L. M. Kelley. 1972. Dermatophyte Test Medium: clinical and quantitative appraisal. *J. Invest. Dermatol.* 58:405-411.
7. Sinski, J. T., J. S. Pokrifchak, and L. M. Kelley. 1975. Quantitative assay of dermatophyte-infected guinea pig skin scales. *J. Clin. Microbiol.* 1:150-153.
8. Taplin, D., N. Zais, G. Rebell, and H. Blank. 1969. Isolation and recognition of dermatophytes on a new medium (DTM). *Arch. Dermatol.* 99:203-209.