JOURNAL OF CLINICAL MICROBIOLOGY, May 1975, p. 465-468

Vol. 1, No. 5
Printed in U.S.A.

Use of Urease Test Disks in the Identification of Mycobacteria

DONNA B. MURPHY AND JEAN E. HAWKINS*

Special Reference Laboratory for Tuberculosis and Other Mycobacterial Diseases, Veterans Administration Hospital, West Haven, Connecticut 06516

Received for publication 26 November 1974

Use of commercially available urea differentiation disks is a rapid and convenient means of determining the urease activity of mycobacteria. In the study performed, paper disks containing Ewing's Urea R Broth were compared with two other methods of testing for urease production. Over 1,500 tests were performed with recent patient isolates from which at least 15 different species of mycobacteria were identified. The tests were read at intervals for 72 h, and a comparison of test results as to rapidity and accuracy for each species was tabulated. The urea disks showed a faster reaction time when compared with the other two methods. They also showed a high percentage (79 to 100%) of reliability for each species on the first run, based on the expected response.

At least a dozen recognized species and complexes of mycobacteria, both potential pathogens and saprophytes, may be encountered with some frequency in the clinical laboratory. The majority can be identified by the performance of selected in vitro tests. Those which do not fit the established schemes of Wayne and Doubek (12) and of Kubica (5) are a significant problem even to large reference laboratories with considerable experience with these organisms.

Tests for urease activity have been reported useful for the identification of mycobacteria (1, 8, 9, 10). The amidase tests of Bönicke (1), although widely used in Europe and Asia, have not been adopted in routine diagnostic laboratories in the United States since they involve time-consuming manipulations of viable cells; and results have not been reproducible in the hand of many workers (10). Less complicated methods for urease have been reported by Toda and associates (8) and Wayne (10). The latter were compared in separate studies with a procedure using commercially available urea-impregnated disks in an attempt to simplify still further the detection of urease activity of mycobacteria.

MATERIALS AND METHODS

Urease tests were performed on over 1,500 strains of mycobacteria representing 15 species. These consisted of primary or recent patient isolates from our own hospital and from submitting hospitals which use us as a reference laboratory. Also examined were well-documented strains from the Trudeau Mycobacterial Culture Collection, Saranac Lake, N.Y. In every run, Trudeau control strains of *Mycobacterium kansasii* (TMC no. 1204), *M. intracellulare* (TMC no. 1406), *M. fortuitum* (TMC no. 1529), and the $H_{37}R_v$ strain of *M. tuberculosis* (TMC no. 102) were included along with a reagent or medium control. The probable identity and expected reaction in the urease test for these organisms were based on criteria established by Wayne and Doubek (12), Kubica (5), and up-dated and summarized in the *Manual of Clinical Microbiology* (7).

Toda's method (8) utilized a 0.01 M phosphate buffer system, pH 6.7, which was autoclaved; while still hot, 3 g of urea and 1 ml of 0.1% phenol red were added per 100 ml. Wayne's procedure (10) employed Difco urea agar base concentrate, according to Christensen's formulation (2) without agar, diluted 1:10 in sterile water. Both substrates were dispensed in 3-ml amounts to sterile borosilicate screw-capped tubes (13 by 100 mm). Individual Difco differentiation disks urea, impregnated with Ewing's Urea R Broth (3), were added to 0.5 ml of sterile water in sterile screw-capped tubes (13 by 100 mm).

Heavy emulsions were made in all three substrate solutions with a spadeful of organisms from a 21-day-old egg base culture and incubated at 35 to 37 C. The results were recorded at 1-h and 24-h intervals for 72 h. Appearance of a cerise color indicated a positive reaction.

RESULTS

The responses observed with the urea disks and Toda's method (8) are shown in Table 1. Of 216 strains of *M. tuberculosis* tested, 213 (98%) were positive with the disks within 24 h. On the other hand, only 185 (85%) reacted by Toda's procedure within 24 h, while 203 (94%) were positive at the end of the incubation period. Of the six strains of *M. kansasii* tested, one failed to react with the disks, but none had reacted by 72 h with the other method. *M. intracellulare-avium* complex and *M. xenopi* were consistently negative by both methods.

With the disks all of the *M. fortuitum* strains tested were positive as expected, but one failed to react by Toda's method.

A similar comparison is shown between the urea disks and Christensen's base concentrate method of Wayne (10; Table 2). The 24-h results show that 254 (94%) of the *M. tuberculosis* strains were positive with the disks, whereas only 199 (74%) were positive by using Wayne's procedure. At 72 h, 263 (98%) were positive by using the disks and 249 (92%) were positive with the other method. In this series, 15 of the 16 *M. kansasii* strains were positive with the disks; however, results with the base were more variable. Again, all strains of the *M. intracellulare-avium* complex, both pigmented and nonchromogenic, and of *M. xenopi* were negative.

TABLE 1. *Comparison of urease reactions by disk method and Toda's method (8)*

Species or complex	No. tested	No. positive (24 H)		No. positive (72 H)	
		Disk	Toda	Disk	Toda
M. tuberculosis	216	213	185	213	203
M. kansasii	6	2	0	5	0
M. gordonae	19	4	1	6	5
M. intracellulare-avium complex					
Nonchromogenic	76	0	0	0	0
Pigmented	24	0	0	0	0
M. xenopi	58	0	0	0	0
M. gastri	6	2	1	5	3
M. terrae complex	9	2	0	2	0
M. fortuitum	10	9	8	10	9

TABLE 2. *Comparison of urease reactions by disk method and Wayne's method (10)*

Species or complex	No. tested	No. positive (24 H)		No. positive (72 H)	
		Disk	Wayne	Disk	Wayne
M. tuberculosis	270	254	199	263	249
M. kansasii	16	6	3	15	6
M. scrofulaceum	13	12	9	13	13
M. gordonae	49	6	5	6	6
M. intracellulare-avium complex					
Nonchromogenic	44	0	0	0	0
Pigmented	18	0	0	0	0
M. xenopi	58	0	0	0	0
M. gastri	3	2	1	2	1
M. terrae complex	6	0	0	0	0
M. fortuitum	6	6	2	6	6
M. chelonei	2	2	2	2	2

M. fortuitum was consistently positive in both methods, but the results were evident earlier with the disks.

A large series of tests has now been carried out by using the urea disks. The total number of tests performed with the disks on individual species is shown in Table 3. The percentages are based on the results achieved on the first run. For the most part, a given species was either distinctly positive or negative. Eighty-six percent or better of strains representing the following species were urease positive: *M. kansasii*, *M. marinum*, *M. scrofulaceum*, *M. szulgai*, *M. fortuitum*, *M. chelonei*, *M. flavescens*, *M. bovis*, and *M. tuberculosis*. Isoniazid-resistant strains of *M. tuberculosis* have been reported to be urease negative (6); but of the 102 strains we tested which were resistant to at least 1 μg of isoniazid per ml, 98 demonstrated urease activity. The majority (84% or greater) of strains of *M. gordonae*, *M. intracellulare-avium* complex, *M. xenopi*, *M. terrae* complex, and *M. triviale* were urease negative.

DISCUSSION

Results reported by Toda et al. (8) were difficult to relate to our findings since many of

TABLE 3. *Usefulness of urea differentiation disks in the characterization of mycobacteria*

Runyon group	Species or complex	No. tested	(%)	
			+	0
	M. tuberculosis	676	98	2
	M. bovis	3	100	0
I	*M. kansasii*	43	91	9
	M. marinum	6	100	0
	M. scrofulaceum	14	100	0
II	*M. gordonae*	108	15	85
	M. szulgai	15	93	7
	M. flavescens	7	86	14
	Intermediate species	13	0	100
	M. intracellulare-avium complex			
	Nonchromogenic	269	1	99
	Pigmented	109	1	99
III	*M. xenopi*	167	0	100
	M. gastri	24	79	21
	M. terrae complex	25	16	84
	M. triviale	4	0	100
IV	*M. fortuitum*	56	100	0
	M. chelonei	28	100	0

the mycobacteria examined had not been speciated. However, in our hands there was good agreement with Toda's data by both his method and the disks on the urease activity of strains of *M. tuberculosis*. On the other hand, in his study all photochromogens gave a positive reaction. Of the six strains of *M. kansasii* tested simultaneously in our laboratory by using both methods, none reacted by Toda's method; but five of the six strains reacted with the disks.

A similar problem was encountered with 16 strains of *M. kansasii* used to compare Wayne's base method with the disks. In our evaluation only six strains (38%) gave a positive reaction with the base, whereas 15 strains reacted with the disks. Wayne reported that all nine of the *M. kansasii* strains that he tested were urease positive (10). However, we were unable to examine these strains in our laboratory since the cultures were no longer available. The reason for this discrepancy is not clear, and repeated tests with additional strains will be needed to establish the reproducibility of the urease reaction of *M. kansasii*.

Of the other species studied concurrently by using the base method and the disks, good correlation was obtained with the exception of *M. gastri*. Only three strains were tested; one gave a positive reaction with the base, while two of the three gave positive disk results. The overall findings with 24 strains of *M. gastri* by using the disks showed that 79% gave a positive reaction in 72 h (Table 3).

Results for the 15 species tested by the disk method showed it to be rapid, sensitive, and convenient, particularly when a small number of tests is run at any given time, as is the case in most clinical laboratories. The low buffer capacity of Ewing's Urea R broth contained in the disks coupled with a larger inoculum size (1 spadeful per 3 ml of Toda's or Wayne's medium, compared with the same inoculum for 0.5 ml of "disk medium") may account for the detection of lesser amounts of ammonia and shorter reaction time. Based on other taxonomic properties, these factors did not appear to produce false positive reactions.

With the disk method no prepared media must be kept on hand. The disks should be added to tubes of sterile water just prior to use in the numbers required. Caution should be exercised in using disposable glassware for the urease test. Non-specific color changes were observed in uninoculated media when some brands of disposable tubes were employed, but was not a problem with multiple-use tubes.

The urease test has also proven to be of value in the study of some scotochromogenic organisms which may present a problem in identification (11). Chromogenic members of the *M. intracellulare-avium* complex are usually described as developing pigment late in the course of incubation. In the present study, some initially scotochromogenic mycobacteria, often deeply pigmented, were biochemically identical to the classical nonchromogenic or late pigment producing members of this complex. These organisms may cause confusion since they appear to differ from *M. scrofulaceum* only in terms of their low catalase activity and ability to reduce tellurite. Kilburn and associates have reported the tellurite reduction test to be useful in differentiating *M. intracellulare-avium* complex from other slowly growing mycobacteria (4). However, in our experience this test has given inconsistent results and has been difficult to read. The urease test seems then to be helpful as an additional basis for differentiation of *M. scrofulaceum* from pigmented strains of the *M. intracellulare-avium* complex. Of 109 pigmented strains identified biochemically as members of the latter complex, 21 were submitted to Werner B. Schaefer for serotyping. Of these, six were identified serologically as members of the *M. avium* complex. The remaining 15 failed to react with any of W. B. Schaefer's antisera..

The 13 "intermediate species" (Table 3) had the following characteristics: scotochromogenic, high catalase activity (>45 mm foam), Tween(R) hydrolysis negative, nitrate reductase negative, and urease negative. They had been isolated from 11 patients, 9 from sputum, 3 from urine, and 1 from a bronchial washing. Eight of these strains were sent to Werner B. Schaefer and two to Emanuel Wolinsky for serotyping, but none of them agglutinated specifically with *M. avium-M. intracellulare-M. scrofulaceum* complex antisera.

From the results of this study, it appears that simple urease tests can be employed on a screening basis in the mycobacteriology laboratory to determine discrete, characteristic responses as an aid to speciation. Comparisons of urea differentiation disks with two other methods have demonstrated the shorter reaction time, more consistent separation into positive and negative responses by species, and the overall convenience and practicality of the disks.

ACKNOWLEDGMENTS

We are grateful to Werner B. Schaefer and Emanuel Wolinsky who attempted to serotype the intermediate species. We thank the staff of the Special Reference Laboratory for their excellent technical assistance. We are also indebted

to Wendy M. Gross for many helpful suggestions during preparation of the manuscript.

LITERATURE CITED

1. Bönicke, R. 1962. Identification of mycobacteria by biochemical methods. Bull. Int. Union Tuber. **32**:13-68.
2. Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella*. J. Bacteriol. **52**:461-466.
3. Edwards, P. R., and W. H. Ewing. 1962. Idenfification of enterobacteriaceae. Burgess Publishing Co., Minneapolis.
4. Kilburn, J. O., V. A. Silcox, and G. P. Kubica. 1969. Differential identification of mycobacteria. V. The tellurite reduction test. Am. Rev. Resp. Dis. **99**:94-100.
5. Kubica, G. P. 1973. Differential identification of mycobacteria. VII. Key features for identification of clinically significant mycobacteria. Am. Rev. Resp. Dis. **107**:9-21.
6. Oka, S., S. Yamaguchi, K. Oizumi, and S. Kudo. 1963. Urease test for isoniazid-resistant human tubercle bacilli. Am. Rev. Resp. Dis. **87**:286-288.
7. Runyon, E. H., A. G. Karlson, G. P. Kubica, and L. G. Wayne. 1974. *Mycobacterium*, p. 148-174. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
8. Toda, T., Y. Hagihara, and K. Takeya. 1961. A simple urease test for the classification of mycobacteria. Am. Rev. Resp. Dis. **83**:757-761.
9. Urabe, K., and H. Saito. 1964. The urease activity of mycobacteria. Am. Rev. Resp. Dis. **90**:266-267.
10. Wayne, L. G. 1974. Simple pyrazinamidase and urease tests for routine identification of mycobacteria. Am. Rev. Resp. Dis. **109**:147-151.
11. Wayne, L. G., T. M. Dietz, C. Gernez-Rieux, P. A. Jenkins, W. Käppler, G. P. Kubica, J. B. G. Kwapinski, G. Meissner, S. R. Pattyn, E. H. Runyon, K. H. Schröder, V. A. Silcox. A. Tacquet, M. Tsukamura, and E. Wolinsky. 1971. A co-operative numerical analysis of scotochromogenic slowly growing mycobacteria. J. Gen. Microbiol. **66**:255-271.
12. Wayne, L. G., and J. R. Doubek. 1968. Diagnostic key to mycobacteria encountered in clinical laboratories. Appl. Microbiol. **16**:925-931.