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Urease Testing of Mycobacteria with BACTEC Radiometric Instrumentation

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A total of 140 mycobacterial isolates from patients treated at Fitzsimons Army Medical Center or the National Jewish Hospital and Research Center and from animal specimens submitted to the National Veterinary Services Laboratory were tested by using a urease procedure modified for use with a BACTEC model 301. Mycobacterial suspensions were prepared by using Middlebrook 7H10 Tween broth. Of the 98 mycobacteria isolates which were urease positive utilizing standard methodology, all were positive using the radiometric procedure. Similarly, all 42 urease-negative isolates were also negative employing the new methodology. Although maximum radiometric readings were observed at 48 h, all positive strains were readily identified 24 h after inoculation without sacrificing either test sensitivity or specificity. Thus, urease testing of mycobacteria, using the modified BACTEC radiometric methodology, was as sensitive, as specific, and more rapid than conventional methods.

Conventional urease testing of mycobacteria requires a heavy inoculation from plate media to a urease broth (2, 4–6). The test broth is then incubated at 35 to 37°C and observed for a color change for up to 72 h. Since the test inoculum requires a large number of organisms, it is frequently necessary to subculture the primary isolate for 4 to 6 weeks before initiation of testing. This procedure appears unnecessarily protracted, particularly in view of the findings of Cox et al. (1) that urease activity can be determined by employing liquid scintillation; however, their procedure also requires subculture of organisms on a solid medium and the use of a scintillation counter, the latter of which is often unavailable in many clinical microbiology laboratories. Since the BACTEC radiometric instrumentation (Johnston Laboratories, Cockeysville, Md.) is currently employed to perform serum gentamicin assay based upon the inhibition of urease activity of a *Proteus* species, it appeared feasible that this instrument could be employed to perform urease testing of mycobacteria. Therefore, we attempted to rapidly grow the various mycobacterial species by using Middlebrook 7H10 Tween broth (M7H10-T) and added this suspension directly to a [^{14}C]urea substrate. A BACTEC model 301 was then used to detect urease activity.

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MATERIALS AND METHODS

Test organisms. One hundred and forty isolates representing 16 mycobacterial species were tested with BACTEC radiometric instrumentation to determine their urease activity. One hundred and thirty of the isolates were obtained from patients treated at Fitzsimons Army Medical Center, Aurora, Colo. or the National Jewish Hospital and Research Center, Denver, Colo. The remaining 10 isolates of *Mycobacterium bovis* were obtained from animal sources and supplied by The National Veterinary Services Laboratory, Ames, Iowa. All cultures were stored at −70°C in M-7H10-T. M-7H10-T was prepared de novo as described for Difco agar (Middlebrook 7H10); however, the formulation was modified to exclude glycerol, malachite green, and agar and supplemented with 0.5 mg of Tween 80 and 1 μg of calcium pantothenate per ml.

Radiometric test solution. The radiometric test solution was prepared by mixing 50.4 ml of aqueous 0.908% potassium monobasic phosphate (KH_2PO_4) with 49.6 ml of aqueous 0.947% anhydrous sodium dibasic phosphate (Na_2HPO_4) and adjusting the pH of the final solution to 6.8 to 7.0. This phosphate buffer solution was dispensed in 2-ml samples into 10-ml Wheaton serum bottles and autoclaved at 121°C for 15 min. After autoclaving, all bottles were sealed with sterile, standard rubber diaphragms and aluminum caps. All bottles were then incubated at 37°C for 24 h and, if sterile, stored at 2°C until required for testing.

Test procedures. The 140 test isolates were initially cultured by using Middlebrook 7H11 agar and tested with standard mycobacterial urease procedures (5). With the completion of this testing, all isolates were transferred to M-7H10-T broth and stored at −70° until required for radiometric urease testing. This later test

procedure was initiated by thawing the frozen isolates at room temperature and transferring 0.2 to 5 ml of fresh M-7H10-T. The lightly inoculated broths were then incubated at 37°C, with agitation at 80 to 100 rpm. When the density of the culture approximated a no. 2 or 3 McFarland standard (McF) (ca. 2 to 10 days), cultures were removed from the incubator, and their densities were adjusted to equal a no. 2 McF by using M-7H10-T. If the suspension appeared clumped, sterile 2-mm glass beads were added to the sample and vigorously agitated on a Vortex mixer to produce a smooth suspension. If necessary, the turbidity of the homogenized culture was then readjusted to equal a no. 2 McF.

One-tenth of a milliliter of this suspension was inoculated into a 10-ml serum vial containing the radiometric test solution. Next, each vial received 0.1 ml of a sterile, aqueous solution containing 20 μCi of [^{14}C]urea (CFA-41, Amersham Corp., Arlington Heights, Ill.) per ml, producing a final concentration of 2 μCi of [^{14}C]urea per bottle. Positive and negative controls were included with each test series. The negative control vial contained all components except microorganisms. The positive control consisted of a suspension of *Proteus mirabilis* standardized and inoculated in the same manner as the test organisms; however, the *P. mirabilis* was prepared and standardized in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) rather than in M-7H10-T. Controls and samples were incubated at 37°C with agitation at 80 to 100 rpm. All bottles were read at 24, 48, and 72 h with a BACTEC model 301, and the growth index (G.I.) readings were recorded. A G.I. reading of 100 is equivalent to 0.025 μCi of ^{14}C released as CO_2.

Test interpretation. As a result of preliminary studies, positive test reactions were determined by subtracting the G.I. reading of the negative control from the test sample reading. If the difference between the two readings was greater than 50, the urease test was considered positive. Readings having differences of 50 or less were considered negative. G.I. readings greater than 1,000 were computed as 1,000 to facilitate the computation of test means. After 72 h all bottles were subcultured onto 5% sheep blood agar plates to insure that the results obtained were not affected by nonmycobacterial contamination. After we completed this quality control procedure, testing was concluded.

RESULTS AND DISCUSSION

The use of M-7H10-T broth permitted rapid cultivation of test strains. It required only 7 to 12 days to obtain sufficient growth of *M. tuberculosis* and *M. bovis* before radiometric urease testing. In contrast, Middlebrook 7H11 agar required an incubation period of 4 to 6 weeks before sufficient growth was achieved to initiate standard urease test procedures. The remaining mycobacteria included in the study also demonstrated more rapid growth with M-7H10-T broth, and it was possible to obtain heavy growth in one-third to one-half the time required when Middlebrook 7H11 agar was employed.

Table 1 summarizes the radiometric urease test data. Of the 16 mycobacterial species included in this study, 12 (98 isolates) are reported to be urease positive, and 4 are urease negative (42 isolates). All isolates used in this investigation gave standard urease test results which agreed with the literature (3, 5). Similarly, all of the isolates examined by using the radiometric methodology also completely agreed with the literature. The average daily G.I. readings for urease-positive mycobacteria were 648, 681, and 631, and the urease-negative strains had average daily readings of 15, 12, and 10 at 24, 48, and 72 h, respectively.

Urease-positive isolates demonstrated peak urea degradation at 48 h and then subsequently declined. Urease-negative strains, on the other hand, demonstrated either consistently low or steadily declining G.I. readings. Although maximum readings were observed at 48 h of incubation, the identification of urease-positive organisms could be made as early as 24 h. All strains positive at 48 h were also strongly positive at 24 h; urease-negative mycobacteria were also easily identified at 24 h. However, standard urease testing required incubation for 72 h before the determination of negative urease activity. Although it did not appear essential for the G.I. reading of the negative control to be subtracted from the test strain, this procedure is still recommended because it assists in compensating for unknown variables in the test system and for possible nonspecific decomposition of the radioactive substrate.

Old or debilitated cultures had erratic urease activity. The use of freshly propagated cultures which were clump free and standardized to a density of a McF no. 2 ensured consistent results. Although this study employed *P. mirabilis* and an uninoculated serum vial as the respective positive and negative controls, routine clinical testing should employ urease-positive and -negative mycobacteria as controls. Since contamination cannot be determined by visual inspection of broth cultures, the contents of all vials should be subcultured to nonselective solid media to ensure that the results are not affected by contaminating microorganisms.

Radiometric urease testing with M-7H10-T appears to be simple, accurate, and rapid means of determining mycobacterial urease activity. It may be employed to facilitate differentiation of *M. avium-intracellulare* and *M. scrofulaceum* (*MAIS* complex) as well as other pathogenic mycobacteria. In addition, more prompt identification of non- or lowly pathogenic mycobacteria such as *M. gordonae, M. gastri, M. terrae*, and *M. triviale* may be expected. Furthermore, the testing may be automated with BACTEC models 5, 225, or 460. It is hoped that when this methodology is fully implemented in conjunction with existing and developing technology, it will pro-

TABLE 1. Urease test results

Culture	No. of isolates	Test results at: 24 h G.I.	24 h % Positive	48 h G.I.	48 h % Positive	72 h G.I.	72 h % Positive
Uninoculated control	12	15[a] (10–19)[b]	0	18 (14–20)	0	17 (12–20)	0
Positive control	12	940 (700–1,000)	100	976 (880–1,000)	100	889 (560–1,000)	100
M. tuberculosis-S[c]	20	726 (100–990)	100	743 (340–1,000)	100	653 (240–1,000)	100
M. tuberculosis-R[d]	10	550 (300–800)	100	606 (500–850)	100	380 (250–500)	100
M. bovis	10	502 (100–1,000)	100	597 (160–1,000)	100	640 (80–1,000)	100
M. kansasii	10	573 (320–1,000)	100	580 (480–1,000)	100	528 (90–1,000)	100
M. marinum	5	346 (280–440)	100	450 (360–600)	100	413 (310–550)	100
M. simiae	3	440 (200–500)	100	600 (375–800)	100	580 (300–800)	100
M. scrofulaceum	5	396 (130–1,000)	100	468 (240–1,000)	100	448 (150–1,000)	100
M. szulgai	5	451 (360–520)	100	576 (390–780)	100	578 (220–800)	100
M. flavescens	3	920 (800–960)	100	>1,000 (e)	100	980 (940–1,000)	100
M. gordonae	10	17 (12–22)	0	13 (8–17)	0	9 (3–12)	0
M. xenopi	7	11 (6–16)	0	14 (10–16)	0	12 (8–14)	0
M. avium-intracellulare	20	20 (18–22)	0	14 (12–20)	0	9 (5–16)	0
M. gastri	5	573 (390–860)	100	480 (390–600)	100	373 (120–650)	100
M. terrae	5	13 (10–22)	0	9 (6–10)	0	8 (3–12)	0
M. fortuitum	10	695 (380–1,000)	100	625 (420–1,000)	100	572 (130–1,000)	100
M. chelonei	10	603 (290–1,000)	100	466 (300–1,000)	100	427 (120–1,000)	100
M. smegmatis	2	>1,000[e]	100	>1,000[e]	100	>1,000[e]	100

[a] Mean radiometric growth index units.
[b] Range radiometric growth index units.
[c] *M. tuberculosis* sensitive to (μg/ml): isoniazid, 1.0; streptomycin, 2.0; ethambutol, 7.5; capreomycin, 5.0; *p*-aminosalicylic acid, 2.0; ethionamide, 5.0; kanamycin, 5.0; and rifampin, 0.5.
[d] *M. tuberculosis* resistant to: one or more of the agents listed above.
[e] Range, all values greater than 1,000.

vide data essential for the prompt, efficacious treatment of patients suffering from mycobacterial infections.

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