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NOTES

Rapid Urease Test for Mycobacteria: Preliminary Observations

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A rapid and sensitive urease test for mycobacteria utilizing a cumulative radiometric technique is described. Definitive results were obtained within a 30-min incubation period. The procedure is simple and economical. Technical time involved is no greater than in conventional procedures.

The determination of urease activity is an integral part of any scheme for the species identification of mycobacteria. Several urease tests for mycobacteria have been described (2, 4, 5) and are dependent upon a change in color of an indicator dye due to the formation of ammonia. These tests require 1 to 3 days of incubation for the detection of positive reactions and thus 3 days of incubation for confirmation of negative reactions. The current work was initiated to develop a rapid and sensitive method of urease testing for mycobacteria.

Conventionally identified (2, 3, 5) clinical isolates and stock cultures (College of American Pathologist survey isolates) of mycobacteria were utilized as the test organisms. The test procedure used the liquid scintillation counting technique described by Buddemeyer (1). Briefly, Whatman no. 40 paper was cut into 5- by 10-cm strips and dipped in Aquasol (New England Nuclear Corp.), and the solvent was allowed to evaporate. Liquid scintillation vials (Rochester Scientific no. 7494 plastic vials) were lined with the fluor-impregnated paper strips and stored at room temperature. Prior to use, 0.3 ml of 1 N NaOH was pipetted onto the impregnated filter strips, and sterile inner sample vials (Rochester Scientific no. 7499) were inserted into the fluor-containing vials. One microcurie of [^{14}C]urea (0.2 mCi/1.4 mg; New England Nuclear Corp.) contained in 0.1 ml of distilled water and 0.5 ml of 0.1 M phosphate buffer, pH 6.7, were pipetted into each inner vial. Stock isotope and buffer were filter sterilized and stored at 4°C. Small inocula of 2- to 4-week-old mycobacteria grown on Lowenstein-Jensen slants were transferred to the inner vials with a 3-mm loop, and the outer vials were tightly capped and incubated at 35°C for 30 min.

Known isolates of mycobacteria representing various groups were tested for urease activity, and the results of these tests are given in Table 1. Attempts were made to transfer similar amounts of inocula for each urease determination. Urease-negative mycobacteria gave counts of less than 40 cpm, whereas the lowest values observed for urease-positive organisms was greater than 3,000 cpm during the 30-min incubation. The mean value for the urease-positive organisms tested was 8,113 cpm during the 30-min incubation, and it was 16 cpm for the negative organisms. The urease-negative isolates were recounted at 30 h of incubation, and counts were less than 100 cpm. A minimum

TABLE 1. *Urease activity of various mycobacterial isolates after 30 min of incubation with [^{14}C]urea*

Organism	cpm	Interpretation
Mycobacterium tuberculosis	3,595	+
M. tuberculosis	12,593	+
M. tuberculosis	3,382	+
M. tuberculosis	7,268	+
M. bovis	10,849	+
M. kansasii	4,890	+
M. kansasii	11,342	+
M. marinum	18,797	+
M. scrofulaceum	4,504	+
M. gordonae	7	−
M. gordonae	5	−
M. szulgai	5,497	+
M. intracellulare	10	−
M. intracellulare	22	−
M. terrae	13	−
M. xenopi	38	−
M. chelonei	6,524	+

75-fold difference in radioactivity was observed between urease-positive and urease-negative organisms. All isolates gave identical urease patterns with both radiometric and conventional methods. Variability of urease activity for certain species of mycobacteria has been reported (2, 5); therefore, greater in-depth studies are needed to determine any range of variability for isolates tested radiometrically. For those laboratories with access to a liquid scintillation counter, a rapid and sensitive urease determination may be made. The procedure is simple and economical. Technical time involved is no greater than the conventional procedures, and the isotope cost was less than 20 cents per test.

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