

NOTES

Preparation of a Stable Mycobacterial Tween Hydrolysis Test Substrate

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A Tween 80 hydrolysis test substrate was found to be stable at room temperature for at least 6 months.

Since its introduction by Wayne (2), the Tween 80 hydrolysis test has found wide application in the differential identification of the mycobacteria. Because of the instability of the substrate, it is necessary to prepare it fresh every 2 weeks (1, 3). In this communication, we describe a method of preparation of a substrate that remains stable for at least 6 months.

A concentrated salt solution was prepared by mixing 38.9 ml of stock solution I to 61.1 ml of stock solution II. Stock solution I was made up by dissolving 22.7 g of KH_2PO_4 in 400.0 ml of distilled water; stock solution II was made up by dissolving 23.8 g of Na_2HPO_4 in 400.0 ml of distilled water. For the substrate, 20.0 mg of neutral red (the percentage of dye content was adjusted to give 1.0 mg of neutral red per ml) was dissolved into 20.0 ml of the concentrated salt solution. A 5-ml portion of Tween 80 (Atlas Powder Company, Wilmington, Del.) was dissolved (with continuous stirring) into the neutral red concentrated salt solution. This substrate was then sterilized by autoclaving and was stored in dark bottles at room temperature.

In the test, two drops of the concentrated substrate were added by capillary pipette to 1.0 ml of sterile, distilled water contained in 13 by 100 mm screw-capped test tubes. Then, a loopful or spadeful of organisms was emulsified into the diluted substrate and the cap was applied. The reaction mixture was then incubated at 37 C. A positive test was indicated by a color change of amber to pink after 10 days of incubation.

At various intervals after preparation, the substrate prepared as described above was used in the Tween hydrolysis test, and the results were compared with those obtained when conventional substrates were used in the test (1, 3). The data depicted in Table 1 were obtained

TABLE 1. Tween 80 hydrolysis test results for various species of mycobacteria on conventional and concentrated substrates

Species	No. of cultures tested	No. of positive cultures ^a	
		Conventional procedure	Concentrated substrate
<i>M. avium-M. intracellulare</i>	10	0	0
<i>M. fortuitum</i>	3	2	2
<i>M. gordonae</i>	10	10	10
<i>M. kansasii</i>	10	10	10
<i>M. scrofulaceum</i>	10	0	0
<i>M. terrae</i>	9	8	8
<i>M. tuberculosis</i>	9	7	7

^a Readings were taken after 10 days of incubation.

with a concentrated substrate prepared 6 months before use and show that the result obtained with this substrate were identical to those obtained by the conventional procedure. The method here described was adopted for routine use in this laboratory, and the concentrated substrate is now commercially available from Difco Laboratories, Detroit, Mich.

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LITERATURE CITED

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