Rifampin Disks in Mycobacterial Susceptibility Testing

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A comparison of rifampin susceptibility test results on 407 strains of mycobacteria using conventional medium in parallel with disk medium showed good agreement. Techniques are described for utilization of drug-impregnated disks in the preparation of medium and for a quality control screening procedure for disks.

The increasing use of rifampin in initial and retreatment regimens for tuberculosis and other mycobacterial infections has resulted in many requests for rifampin susceptibility tests. In preliminary experiments to establish procedures for preparation of conventional rifampin media, dimethylformamide was chosen as a solvent for rifampin (9). Its solubilization capacity permitted a lower concentration of solvent in the final products than solvents used by others (5, 8). Each time rifampin media were prepared, an individually weighed portion of powdered drug was solubilized with precautions against the toxicity of the solvent and then diluted immediately before use. Previously published methodology and data (2, 3, 10) have shown that paper disks impregnated with other anti-tuberculosis drugs are convenient and satisfactory for the preparation of media suitable for the susceptibility testing of mycobacteria by recommended procedures (6). By diffusion, the disks supply drugs into measured amounts of agar in place of the conventional method using fluid drug solutions mixed into molten agar. Properly prepared disk media may be substituted for conventional media in all phases of mycobacterial susceptibility testing, provided that the drugs in question diffuse adequately under specified conditions and that the lots of disks in use contain appropriate amounts of drug as demonstrated by their ability to yield results comparable to conventional media. The purpose of this study was to evaluate drug susceptibility testing medium made with rifampin-impregnated disks by comparing the results of parallel tests using medium prepared with disks and medium prepared conventionally.

The method of preparing drug medium for mycobacterial tests using disks involves placing a disk in the center of an empty petri dish quadrant, adding 5 ml of sterile 7H10 medium with a device such as a Cornwall pipetter (Becton, Dickinson & Co., Rutherford, N.J., no. 3056), repositioning the disk if necessary with the delivery tip of the pipetter, and incubating the medium for 24 h at 35 C to permit some diffusion of drug from the disk into the medium before inoculation (2, 3).

In the recommended mycobacterial drug susceptibility testing procedure, one control quadrant of drug-free medium per dish and three quadrants of drug media are inoculated with dilute suspensions of the organism under test and incubated for 21 days at 35 C in an aerobic atmosphere enriched with approximately 10% carbon dioxide. Colonies are then counted, and those "resistant" colonies appearing on the drug media are reported as a percentage of the colonies on the adjacent control quadrant (6). A minimum of 100 colonies is desired on the control quadrant, and serial dilutions of organisms or smear-positive processed specimens are inoculated on successive petri dishes so that the numbers of colonies on quadrants with heavy growth may be estimated by reference to the next dilution. If the drug medium produces no growth or colonies fewer than 1% of the colonies on the control medium, the in vitro interpretation is "susceptible." If the number of colonies on drug medium equals 1 to 10% of the control growth, the usual interpretation is "partially resistant." Growth equal to 11% or more of the growth on control medium indicates in vitro resistance, and is reported in ranges of 11 to 20%, 21 to 30%, 31 to 40%, 41 to 50%, and over 50%.

In this investigation the media for comparison were placed in separate compartments of the same dish to ensure identical handling. Each dish contained one quadrant of 5 ml of control medium. The second quadrant contained 5 ml of medium containing 1 μg of rifampin.
rifampin per ml prepared in the conventional manner: powdered rifampin (Dow Chemical Co., Indianapolis, Ind., lots 1E5W, 2BIR, and 3A4C) was solubilized in N,N-dimethylformamide (Aldrich Chemical Co., Milwaukee, Wis., lot 020907) at a concentration of 50 mg of active rifampin per ml, diluted in warm sterile distilled water to 100 μg/ml, added immediately to the medium in a ratio of 1:100 to give 1 μg/ml, and then mixed on a magnetic stirrer before dispensing. The third quadrant contained a 5-μg rifampin disk (BBL, Cockeysville, Md., RA5 Sensi-discs, lots 106047 and 301026) submerged in 5 ml of medium. Initially the fourth quadrant contained a drug-free solvent control of dimethylformamide diluted 1:50,000 in the medium until it was confirmed that this concentration of solvent, which was also present in the conventional rifampin medium, had no observable effect upon mycobacterial growth. All quadrants in the same dish received their medium from the same flask of autoclaved and enriched 7H10 medium prepared from A.C.S. reagent glycerin (Allied Chemical Corp., Morristown, N.J., B & A code 1782, lots D174 and E201), dehydrated agar base and fluid enrichment (Difco Laboratories, Detroit, Mich., Middlebrook 7H10 agar, control numbers 536270 and 551509; and Middlebrook OADC enrichment, control numbers 557729, 564092, 570144, 570469, 585470, and 588949). After 24 h at 35°C, the media were inoculated or stored no more than 2 weeks at 4°C before inoculation.

Parallel tests on conventional and disk media were performed on 407 strains of mycobacteria. Figure 1 shows a comparison of results from each type of medium. No major discrepancies were found in the entire series which would have resulted in a "susceptible" interpretation of the results from one medium and a "resistant" interpretation from the other medium. Six minor discrepancies involving the "partially resistant" 1 to 10% range were found.

The test results by species are listed in Table 1. In agreement with other reports (1, 4, 5, 7, 8, 11) a majority of the Mycobacterium tuberculosis, Mycobacterium kansasii, Mycobacterium marinum, and Mycobacterium gordoneae strains tested were "susceptible" and a majority of the Mycobacterium scrofulaceum, Mycobacterium

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<th>Resistance (%)</th>
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**Fig. 1.** Comparison of 407 mycobacterial susceptibility test results on disk and conventional media containing 1 μg of rifampin per ml of 7H10 medium. Entries show the number of strains producing the indicated percentage of rifampin-resistant colonies on each rifampin medium as compared with drug-free control medium. S, PR, and R give in vitro interpretations of susceptible, partially resistant, and resistant. Shaded boxes indicate areas of discrepancy between "susceptible" and "resistant" results.
have not yet been found. Furthermore, studies consisting of parallel testing such as the one being reported here are clearly impractical to perform on all successive lots of disks received. A simpler approach is to prepare conventional drug media in three concentrations: the exact amount of drug which the disk medium should contain, the lowest concentration of drug which would be acceptable for use, and the highest acceptable concentration. Replicate dishes of these media should be tested under routine test conditions in parallel with disk medium, utilizing a few cultures which show intermediate resistance in colony size or colony numbers on routine drug medium. The strains chosen must respond differently to the highest and lowest drug concentrations used to be suitable. In practice, if the three concentrations of conventional drug medium are set at 67, 100, and 150% of the concentration desired for routine use, then the lowest and highest concentration will coincide with the limits specified by the Food and Drug Administration for most antimicrobial disks. This quality control screening procedure does not provide a precise assay of drug content, but it does help the user determine whether a particular lot of disks will give acceptable results in the recommended susceptibility test procedure for mycobacteria.

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LITERATURE CITED

