JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1554–1559
0095-1137/94/$04.00+0

Vol. 32, No. 6

Antimicrobial Susceptibilities of Mycobacteria as Determined by Differential Light Scattering and Correlation with Results from Multiple Reference Laboratories

PATRICIA S. CONVILLE,* FRANK G. WITEBSKY, AND JAMES D. MACLOWRY†

Microbiology Service, Clinical Pathology Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland 20892

Received 29 October 1993/Returned for modification 22 December 1993/Accepted 21 March 1994

The DAWN Model B laser light scattering instrument (Wyatt Technology Corporation, Santa Barbara, Calif.) was evaluated to assess its potential to provide rapid mycobacterial antimicrobial susceptibility test results. For *Mycobacterium tuberculosis* there was a clear separation between susceptible and resistant results with the isolates tested, and there was excellent correlation with reference laboratory results. For *Mycobacterium avium* there was no obvious breakpoint between susceptible and resistant results with the isolates tested, and correlation with reference laboratory results was less good than for *M. tuberculosis*. However, for *M. avium* there was also less agreement among reference laboratory results than for *M. tuberculosis*. Significant instrument design and software program changes would be required for the instrument to become a useful tool for mycobacterial susceptibility testing in the diagnostic laboratory.

The "modified proportion method" traditionally used in the United States for susceptibility testing of isolates of *Mycobacterium tuberculosis* requires 3 weeks before final results can be obtained (7). The BACTEC 460 System (Becton Dickinson, Sparks, Md.) provides a significant improvement over the traditional method, in that results are generally available in less than 10 days (2). As a consequence of the rising incidence of multidrug-resistant *M. tuberculosis* isolates, the need for rapid and accurate determination of the antimicrobial susceptibilities of *M. tuberculosis* isolates is being increasingly emphasized (8). Rapid determination of the susceptibilities of other slowly growing mycobacteria, particularly of members of the *Mycobacterium avium* complex, might also be useful, although the clinical relevance of in vitro results remains uncertain (4). While the recent development of a procedure utilizing a phage-delivered luciferase reporter gene (5) may eventually allow mycobacterial susceptibility determinations to be completed even more rapidly than with the BACTEC system, the luciferase-based technique is not yet available for routine use in mycobacteriology laboratories. Previous studies have suggested that differential light scattering measurements may be useful for the rapid detection of some of the effects produced by antimicrobial agents (1, 6). The DAWN Model B (Wyatt Technology Corporation, Santa Barbara, Calif.) is a later-generation instrument designed to measure the differential light scattering produced by particulate suspensions exposed to vertically polarized laser light. We evaluated this instrument to assess its potential for use in rapid mycobacterial susceptibility testing and to determine if it might offer any advantages over the BACTEC system.

MATERIALS AND METHODS

Instrument. The DAWN Model B is a light scattering photometer utilizing a vertically polarized helium-neon laser as the light source. A narrow-diameter beam (wavelength 632.83 nm) is directed through a sample placed in the read head. Fifteen photodiode detectors arranged in set angles from 35 to 135° forward scattering around the sample measure the intensity of the light scattered by particles in the sample. One hundred readings are collected from each detector and the laser monitor, usually within 4 to 8 s. The ten lowest values per detector are then used for sample analysis, thereby eliminating high readings caused by interfering particles and/or noise spikes.

The DAWN-B87 software package written in DOS allows for the collection, recording, and manipulation of the scattering data. Background readings from the detectors and from the solvent are subtracted from sample readings by this program. A variety of averaged and calculated values are reported for each sample. In addition, a differential light scattering curve, which shows the variation in the intensity of the light scattered by particles in the suspension as a function of the scattering angle, is produced.

Organisms. Six isolates of *M. tuberculosis* were used for this study. Isolates were selected to provide a variety of susceptibility patterns, and isolates with well-documented patterns, such as CAP and ATCC strains, were used when possible. Susceptible and resistant patient strains recovered in this laboratory were also used (Table 1).

Since *M. avium* isolates with well-documented susceptibility patterns were unavailable, five recent clinical isolates, the susceptibility patterns of which had been previously determined by the Centers for Disease Control, were selected for analysis by DAWN.

Solutions of antimicrobial agents. Solutions of antimicrobial agents were prepared at the following concentrations in Dubos Broth Base (Difco, Detroit, Mich.) with a final concentration of 10% Dubos Medium Albumin (Difco): streptomycin (Pfizer, Inc., Groton, Conn.), 16 μg/ml, 8 μg/ml, and 2 μg/ml; isonicotinic hydrazide (Aldrich Chemical Company, Inc., Milwaukee, Wis.), 5 μg/ml, 1 μg/ml, and 0.2 μg/ml; rifampin (Merrell Dow Pharmaceuticals, Cincinnati, Ohio), 10 μg/ml, 5 μg/ml, and 1 μg/ml; and ethambutol (Lederle Laboratories, Pearl River,

* Corresponding author. Mailing address: Microbiology Service, Building 10, Room 2C-385, National Institutes of Health, Bethesda, MD 20892. Phone: (301) 496-4433. Fax: (301) 402-1886.
† Present address: 5800 Conway Rd., Bethesda, MD 20817.

TABLE 1. Source and susceptibility patterns of *M. tuberculosis* isolates

Isolate	Source	Susceptibility testing result			
		Streptomycin	INH[a]	Rifampin	Ethambutol
267	Patient isolate[b]	S[c]	R[d]	S	S
2494	Patient isolate[b]	S	S	S	S
2384	Patient isolate[b]	R	R	R	S
ATCC	Strain ATCC 35837	S	S	S	R
667	CAP 1986 E-8[e]	S	S	R	S
10	CAP 1986 E-13[e]	R	R	S	S

[a] Isonicotinic hydrazide.
[b] Results by radiometric method performed at NIH.
[c] Susceptible.
[d] Resistant.
[e] College of American Pathologists proficiency sample isolate.

N.Y.), 16 μg/ml, 8 μg/ml, and 4 μg/ml. Each solution had a total volume of 4.5 ml.

Preparation of the organism suspensions. A suspension of each organism was prepared in a 15-ml Falcon tube (Becton Dickinson, Lincoln Park, N.J.) containing 10 ml of Dubos broth. The tube was capped and vortexed vigorously after the addition of 3-mm sterile glass beads (Curtin Matheson Scientific, Inc., Jessup, Md.) until large organism clumps were broken and the suspension approximated a 1 McFarland standard. Suspensions were allowed to stand until remaining clumps settled, and the upper portion of each suspension was transferred to another Falcon tube.

One-half milliliter of the organism suspension was added to each antibiotic dilution tube, bringing the total volume in each tube to 5 ml. One-half milliliter of the suspension was also added to two drug-free control tubes containing 4.5 ml of Dubos broth. One control was aliquoted for DAWN testing at 0 h and was not tested further. All other suspensions including the remaining control were incubated with loosened caps at 35°C ± 2°C in 8% CO_2 and were tested on the DAWN after 48 h, 72 h, and 7 days of incubation.

At the time of the 7-day reading, an aliquot from each control and antibiotic-containing tube was subcultured to sheep blood agar (REMEL, Lenexa, Kans.) to check for possible contamination occurring during the course of the experiment.

DAWN readings. Ten milliliters of filter-sterilized distilled water was added to 20-ml glass scintillation vials (Kimball, Toledo, Ohio). Water-containing vials were refrigerated until inoculated. At the time of inoculation, the water vials were allowed to come to room temperature and were inoculated with either 1 ml of the various *M. tuberculosis* drug suspensions or 1 ml of *M. tuberculosis* control, or 0.5 ml of the *M. avium* drug suspensions or 0.5 ml of *M. avium* control. Different inoculum volumes for each species were used to compensate for the varying growth rates of the two organisms. Vials were capped and vortexed and were allowed to stand for at least 5 min before being read.

Using the DAWN-B87 program, dark offset readings were taken (detector background) and a standard water sample reading was entered (solvent background). All vials were read by using the DAWN-B87 program.

DAWN calculations. The log-weighted average intensity (LWAI) is a mathematically derived value calculated from the intensities of scattered light read by all 15 detectors during the testing interval. Compared with other values calculated by DAWN, LWAI appeared to most closely reflect changes in organism growth. Initial studies showed these LWAI values to compare best with reference laboratory results.

In comparing DAWN results with reference methodologies, we used the LWAI delta value, which was calculated as the difference between the 7-day reading and the 0-h reading.

For ease of calculation, samples which were saturated (growth too dense to be read by the instrument) were arbitrarily assigned a LWAI value of 450.

Reference susceptibility methods. Three isolates each of *M. tuberculosis* and *M. avium* were sent to two reference laboratories for conventional susceptibility testing. The Mayo Clinic (Rochester, Minn.) performed agar dilution tests and the National Jewish Center for Immunology and Respiratory Medicine (Denver, Colo.) determined radiometric broth dilution MICs. All five *M. avium* isolates were tested by the Centers for Disease Control using the agar dilution technique. The National Institutes of Health performed radiometric broth dilution susceptibility tests for all *M. tuberculosis* isolates according to the instructions of the manufacturer of the BACTEC 460. Radiometric broth dilution susceptibility tests were also performed by using three of the *M. avium* isolates according to the recommendations of the manufacturer of the BACTEC 460 and by using concentrations comparable to the DAWN concentrations.

RESULTS

Figures 1 and 2 show the changes in LWAI over the course of the 7-day incubation period for representative *M. tuberculosis* and *M. avium* isolates (one of each species) in combination with various concentrations of the drugs tested. For both organisms LWAI values generally remained constant or increased slightly through 48 h of incubation, at which time values began to increase significantly for those concentrations to which isolates are highly resistant, with high values attained by 7 days. Over this time period, LWAI values for concentrations to which isolates are susceptible remained constant at low levels or increased slowly and then decreased or remained constant.

To determine breakpoints in LWAI delta values for *M. tuberculosis* which might correspond to resistance or susceptibility, the DAWN LWAI delta results and the corresponding reference method results of all *M. tuberculosis* isolates were ranked on the basis of the LWAI delta value (data not shown). A large gap in LWAI delta values was observed between 28.8 and 75.5, with no values falling between these two points. We therefore defined delta values of ≤30 as susceptible, and this value was used as the susceptible breakpoint for *M. tuberculosis* for the DAWN methodology.

M. tuberculosis isolates tested at a total of 63 drug concentrations had both a DAWN result and at least one reference method result (Table 2). Fifty of these concentrations were susceptible by all reference methods tested. When the previously described method for determining susceptibility or resistance for *M. tuberculosis* was used, 49 of 50 concentrations (98%) were determined to be susceptible by DAWN. *M. tuberculosis* isolates tested at 11 drug concentrations were resistant by all reference methods tested; 10 of these (91%) were also resistant by DAWN. In two instances reference methods did not agree.

When the *M. avium* isolates were similarly ranked by LWAI delta values, no obvious breakpoint in values was present. Table 3 gives the delta values for the controls and all drug concentrations of each *M. avium* isolate tested. Also listed are the values obtained for each concentration by each reference

TABLE 2. Comparison of LWAI delta values and reference laboratory results for *M. tuberculosis* isolates

Strain	Method	Delta value for growth control	Delta value or susceptibility result for concns of:											
			INH[a]			Ethambutol			Rifampin			Streptomycin		
			0.2 μg/ml	1.0 μg/ml	5.0 μg/ml	4.0 μg/ml	8.0 μg/ml	16.0 μg/ml	1.0 μg/ml	5.0 μg/ml	10.0 μg/ml	2.0 μg/ml	8.0 μg/ml	16.0 μg/ml
267	DAWN delta	134.4	107.9	79.2	0.0	8.2	NT	5.9	−3.0	−3.4	−4.7	−7.9	−3.9	−1.5
	NIH BACTEC		R[b]	NT[c]	NT	S[d]	S	S	S	S	S	NT	S	S
	Agar dilution													
	Broth dilution													
2494	DAWN delta	141.0	−2.7	1.1	−2.6	5.6	4.8	2.9	−6.2	−5.5	−8.8	−2.7	−5.9	−4.2
	NIH BACTEC		S	S	S	S	S	S	S	S	S	NT	S	S
	Agar dilution													
	Broth dilution													
ATCC	DAWN delta	420.1	13.1	13.9	10.6	378.3	339.1	169.3	3.9	5.5	7.4	2.4	−0.2	2.6
	NIH BACTEC		S	S	S	R	NT	NT	S	S	S	S	S	S
	Agar dilution		NT	S	S	R	R	NT	S	S	S	NT	S	S
	Broth dilution		S	S	S	R	S	S	S	S	S	S	S	S
667	DAWN delta	141.6	3.4	4.8	10.3	18.3	14.0	22.8	77.2	91.6	94.6	1.7	−1.1	21.2
	NIH BACTEC		S	S	S	NT	S	S	NT	R	R	S	S	S
	Agar dilution		NT	S	S	S	S	S	R	NT	NT	NT	S	S
	Broth dilution		S	S	S	S	S	S	R	R	R	S	S	S
10	DAWN delta	155.4	120.8	111.5	98.0	28.8	24.6	20.9	−2.7	−4.6	−3.4	173.8	94.1	164.8
	NIH BACTEC		R	NT	NT	S	S	S	S	S	S	R	NT	NT
	Agar dilution													
	Broth dilution													
2384	DAWN delta	210.3	174.2	24.1	0.4	17.3	25.0	20.5	5.2	5.1	3.2	184.5	103.4	75.5
	NIH BACTEC		R	NT	NT	NT	S	S	NT	S	S	R	NT	NT
	Agar dilution		R	R	S	S	S	S	S	S	S	R	R	R
	Broth dilution		R	R	S	S	S	S	S	S	S	R	R	S

[a] Isonicotinic hydrazide.
[b] Resistant.
[c] Not tested.
[d] Susceptible.

method. In order to assign "susceptible" or "resistant" designations to the DAWN results for *M. avium*, various cut-off values were evaluated by comparing DAWN LWAI delta values with the results of reference methods which were in agreement at each drug concentration. Table 4 shows the correlation of DAWN results with reference results obtained with various delta cutoffs, using only those results with complete reference agreement by at least two methods.

M. avium isolates tested at a total of 36 drug concentrations had both DAWN results and at least 2 reference method results (Table 3). Thirteen of these concentrations were susceptible by all reference methods tested and, when a delta value cutoff of 30 (as was chosen for *M. tuberculosis*) was used, 10 of these 13 concentrations (77%) were also susceptible by DAWN (Table 4). *M. avium* isolates tested at seven drug concentrations were resistant by all reference methods tested; all were also resistant by DAWN. With a delta value cutoff of 40, 11 of 13 concentrations (85%) were susceptible by DAWN and all reference methods, while all concentrations resistant by all reference methods were also resistant by DAWN (Table 4). With a delta value cutoff of 50, 12 of 13 concentrations (92%) were susceptible by DAWN and all reference methods, while 6 of 7 concentrations (86%) were resistant by DAWN and all reference methods (Table 4). There were 16 concentrations at which the reference methods did not agree.

DISCUSSION

In the DAWN system, the LWAI value describes the average intensity of the scattered light read by the 15 detectors in the reading head. Of all the values reported by the DAWN software, the LWAI proved to be the most consistently evaluable parameter which reflected changes in organism growth. The LWAI values were generally found to increase from day to day if the organisms were actively growing; the LWAI of resistant organisms increased significantly after the first 48 h of incubation. For susceptible organisms, the LWAI values were found to remain near the 0-h value throughout the incubation period (Fig. 1 and 2).

A version of the LWAI which proved to be especially useful was the LWAI delta value. This was calculated as the difference between the 7-day LWAI value and the 0-h LWAI reading. The delta value gave a clear indication of the extent of growth for each organism-drug combination over the entire incubation period.

With the small number of isolates tested, we cannot unequivocally define a LWAI delta value below which any organism could be considered susceptible or above which it could be considered resistant. However, at least for the *M. tuberculosis* isolates tested in this study, some clear breakpoint values were apparent. When LWAI delta values were ranked for drug-*M. tuberculosis* combinations, the largest break in

TABLE 3. Comparison of LWAI delta values and reference laboratory results for *M. avium* complex isolates

Strain	Method	Delta value for growth control	Delta value or susceptibility result for concns of:											
			INH[a]			Ethambutol			Rifampin			Streptomycin		
			0.2 μg/ml	1.0 μg/ml	5.0 μg/ml	4.0 μg/ml	8.0 μg/ml	16.0 μg/ml	1.0 μg/ml	5.0 μg/ml	10.0 μg/ml	2.0 μg/ml	8.0 μg/ml	16.0 μg/ml
7548	DAWN delta	302.3	55.7	47.5	53.0	50.4	37.8	39.6	1.6	−2.5	−5.5	13.9	5.1	6.4
	NIH BACTEC													
	Agar dilution[e]													
	Broth dilution													
	Agar dilution[f]		R[b]	R	S[c]	R	NT[d]	NT	R	NT	NT	R	NT	S
5372	DAWN delta	319.7	275.2	75.4	58.7	140.5	66.0	46.0	15.9	7.3	5.9	45.0	34.2	16.0
	NIH BACTEC		R	NT	NT	NT	S	S	S	S	S	NT	S	S
	Agar dilution[e]		R	R	NT	R	R	NT	R	S	S	R	R	R
	Broth dilution		R	R	S	S	S	S	R	S	S	R	S	S
	Agar dilution[f]		R	R	R	R	NT	NT	R	NT	NT	R	R	NT
41	DAWN delta	268.9	254.3	80.4	46.8	417.8	132.8	50.1	99.6	88.8	87.8	75.5	59.8	46.7
	NIH BACTEC													
	Agar dilution[e]													
	Broth dilution													
	Agar dilution[f]		R	R	R	R	NT	NT	R	NT	NT	R	R	NT
3555	DAWN delta	426.2	426.2	192.9	51.8	119.9	36.0	31.6	−1.5	0.1	−3.4	8.1	−1.9	0.1
	NIH BACTEC		R	NT	NT	NT	S	S	S	S	S	S	S	S
	Agar dilution[e]		R	R	NT	R	R	NT	R	S	S	NT	S	S
	Broth dilution		R	R	S	S	S	S	S	S	S	S	S	S
	Agar dilution[f]		R	R	R	R	NT	NT	R	NT	NT	R	NT	S
293	DAWN delta	420.9	420.9	64.8	43.3	121.5	75.2	50.8	0.4	−2.4	−3.0	16.7	1.1	−2.3
	NIH BACTEC		R	NT	NT	NT	S	S	S	S	S	S	S	S
	Agar dilution[e]		R	R	NT	R	R	NT	R	S	S	NT	S	S
	Broth dilution		R	R	S	S	S	S	S	S	S	S	S	S
	Agar dilution[f]		R	R	R	R	NT	NT	R	NT	NT	R	NT	S

[a] Isonicotinic hydrazide.
[b] Resistant.
[c] Susceptible.
[d] Not tested.
[e] Test performed at Mayo Clinic.
[f] Test performed at Centers for Disease Control.

values fell between 28.8 and 75.5. This suggests that the breakpoint value for resistance versus susceptibility may lie somewhere between these two values. Because of this gap, we assigned a LWAI value of 30 as the upper limit for susceptible organisms. With the testing of more isolates, LWAI delta values might be found which fall between 30 and 75; this would help to further define the breakpoint value.

Using these criteria, 59 of 61 (97%) of the drug-*M. tuberculosis* combinations evaluated by DAWN were in agreement with the reference methods. One drug-*M. tuberculosis* combination (strain ATCC 35837-ethambutol, 16 μg/ml) was resistant by DAWN and susceptible by broth dilution (not tested by agar dilution or NIH BACTEC), and one drug-*M. tuberculosis* combination (no. 2384, isonicotinic hydrazide, 1 μg/ml) was susceptible by DAWN and resistant by both agar and broth dilution (not tested by NIH BACTEC). Overall, there was a high correlation between DAWN results and reference laboratory results, and the breakpoint value we defined for *M. tuberculosis* appeared to make the interpretation of DAWN data fairly clear-cut.

When the LWAI delta values were similarly ranked for all drug-*M. avium* MA combinations, no obvious breakpoint values were observed. We attempted to arbitrarily define breakpoint values which might allow the best correlation of DAWN and reference methodologies (Table 4). It appeared that for *M. avium*, a cut-off value of 40 gave the best correlation with reference methods, with DAWN results agreeing with reference laboratory results in 90% of the cases. With the use of this cutoff, agreement between DAWN and reference methodologies was 85% for susceptible drug-*M. avium* combinations and 100% for resistant combinations.

An examination of all results obtained with the various testing methodologies revealed the problems which exist in the susceptibility testing of *M. avium*. While the results from all methods of testing *M. tuberculosis* showed a high degree of correlation, there was wide variation in the results obtained from the various methods of testing isolates of *M. avium*. In this study, reference methodologies were in agreement with one another in only 35 of 50 (70%) of the drug-*M. avium* combinations tested. Susceptibility of the *M. avium* complex to antimycobacterial agents has been reported to vary with colony type (3) or with the method used, either broth or agar dilution (3). In this study, isolates sent to the reference laboratories were from the same subculture, yet results obtained by the broth and agar dilution methodologies varied in 9 of the 28 combinations tested (32%). Therefore, inconsistencies in results are more likely to have resulted from procedural variations than from organism variations.

Our goal in designing this evaluation was to obtain susceptibility results as early as possible, hopefully within 72 h. In

TABLE 4. Comparison of susceptible and resistant results for all methods for *M. avium* complex isolates

DAWN LWAI delta cutoff value	No. of isolates susceptible by all reference methods tested[a]	No. (%) of isolates also susceptible by DAWN cutoff	No. of isolates resistant by all reference methods tested[a]	No. (%) of isolates also resistant by DAWN cutoff	Total DAWN agreement (%) with reference methods
30	13	10 (77%)	7	7 (100%)	85
40	13	11 (85%)	7	7 (100%)	90
50	13	12 (92%)	7	6 (86%)	90

[a] At least two reference methods were tested.

some cases, clearly resistant or susceptible values were obvious at 72 h. However, using the criteria we have selected, we think that reporting results at 72 h would be premature. Examination of Fig. 1 and 2 suggests that it might be possible to use delta values covering the 0-to-96-h incubation period to evaluate the susceptibility results.

There are several problems associated with the use of this instrument. The procedure reported here requires extensive transferral of organisms from incubation tubes to reading vials, resulting in a potential exposure hazard and the use of a significant amount of technologist time. There is also a significant risk of introducing contaminating organisms into the incubation tubes during the set-up and transfer processes. This potential for contamination is increased by the cumbersomeness of the scintillation vials used for the DAWN readings and by the special handling constraints necessary when working with mycobacteria. Methodologies involving less organism transfer would eliminate some of the potential exposure and contamination concerns.

The DAWN-B87 software produces numerous averaged and calculated values, many of which are difficult for the laboratorian to interpret and to relate to susceptibility or resistance. In order to make this instrument more useful for susceptibility testing, some software redesign would be helpful, so that changes in program-calculated parameters would more obviously correlate with traditional MIC determinations.

It remains to be determined if a DAWN instrument in routine use would be able to provide mycobacterial susceptibility results significantly faster than the BACTEC system, although the DAWN instrument does have the advantage of not requiring the use of radionuclides. It seems doubtful that even a modified DAWN instrument would have the potential to provide results as rapidly as the luciferase-based techniques, given that the DAWN instrument can test only morphologic changes, while the luciferase-based techniques can detect more subtle biochemical changes.

Our work with the DAWN instrument was designed to be a preliminary evaluation of the instrument and its laser technology as a means of performing rapid mycobacterial susceptibility tests. While the system does appear to have some potential for this use, more extensive evaluations are required to adequately define this potential. It might also be feasible to assess the effects of combinations of drugs with the DAWN instrument, since the test is performed in liquid media. Before the instrument could be a practical tool for the mycobacteriology laboratory, however, the design of the instrument, its software,

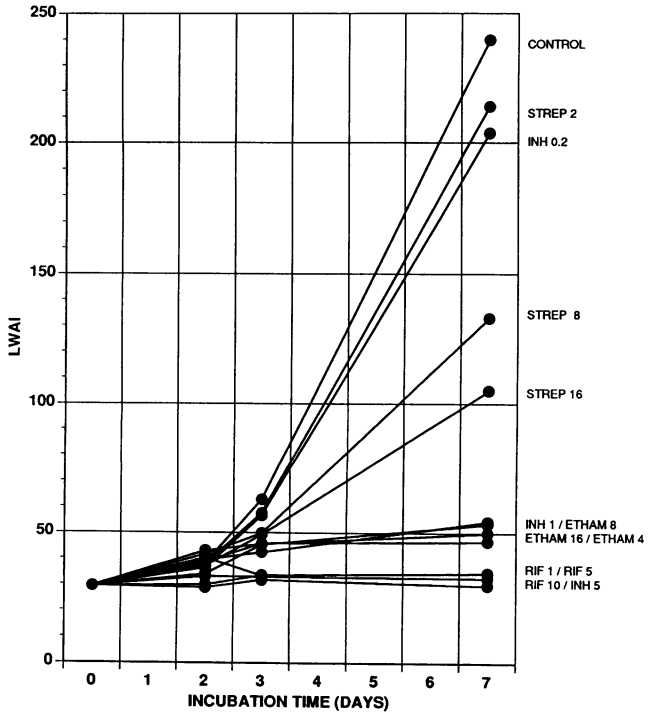

FIG. 1. LWAI versus incubation time for one *M. tuberculosis* isolate.

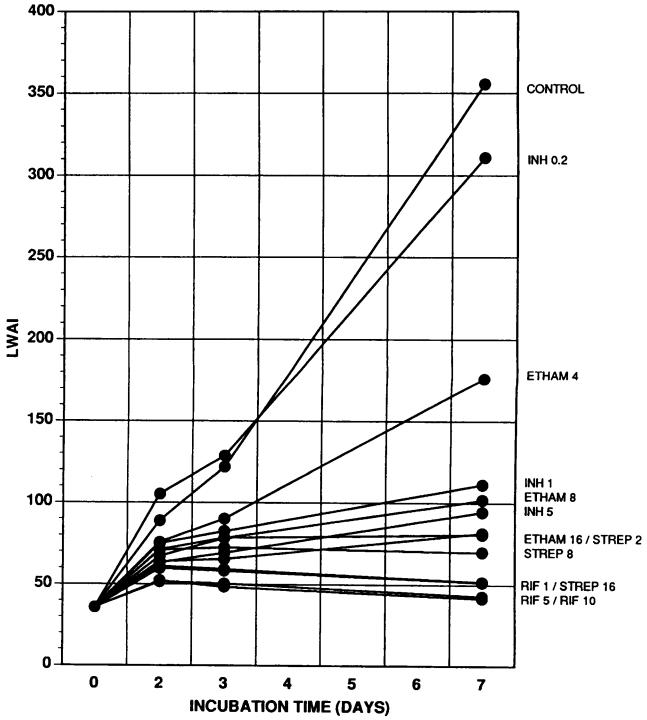

FIG. 2. LWAI versus incubation time for one *M. avium* isolate.

and its testing protocols would require modification to permit fewer labor-intensive and exposure-prone manipulations and easier interpretation of results.

ACKNOWLEDGMENT

We thank Philip J. Wyatt for his assistance with the DAWN instrument and with data analysis.

REFERENCES

1. **Berkman, R. M., P. J. Wyatt, and D. T. Phillips.** 1970. Rapid detection of penicillin sensitivity in *Staphylococcus aureus*. Nature (London) **228:**458–460.
2. **Heifets, L.** 1986. Rapid automated methods (BACTEC system) in clinical mycobacteriology. Semin. Respir. Infect. **1:**242–249.
3. **Heifets, L. B.** 1991. Dilemmas and realities in drug susceptibility testing of *M. avium-M. intracellulare* and other slowly growing nontuberculous mycobacteria, p. 123–146. *In* L. B. Heifets (ed.), Drug susceptibility in the chemotherapy of mycobacterial infections. CRC Press, Inc., Boca Raton, Fla.
4. **Inderlied, C. B., C. A. Kemper, and L. E. M. Bermudez.** 1993. The *Mycobacterium avium* complex. Clin. Microbiol. Rev. **6:**266–310.
5. **Jacobs, W. R., R. G. Barletta, R. Udani, J. Chan, G. Kalkut, G. Sosne, T. Kieser, G. J. Sarkis, G. F. Hatfull, and B. R. Bloom.** 1993. Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. Science **260:**819–822.
6. **Murray, J., P. Evans, and D. W. L. Hukins.** 1980. Light-scattering methods for antibiotic sensitivity tests. J. Clin. Pathol. **33:**995–1001.
7. **National Committee for Clinical Laboratory Standards.** 1990. Antimycobacterial susceptibility testing. Proposed standard M24-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
8. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburg, Jr., and R. C. Good.** 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31:**767–770.