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# Determination of MICs for *Mycobacterium avium-M. intracellulare* Complex in Liquid Medium by a Colorimetric Method

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**We investigated the potential of a rapid colorimetric microassay based on the reduction of dimethylthiazol-diphenyltetrazolium bromide (MTT) for determining the growth of *Mycobacterium avium-M. intracellulare* complex (MAC) and MICs of clofazimine, resorcinomycin A, and the quinolone PD 127391 against MAC. The reduction of MTT was directly proportional to the number of viable bacteria. A comparison of the MTT reduction test with the [$^3$H]glycerol uptake assay showed the former to possess higher analytical sensitivity for detecting MAC growth in microtiter plates. The MTT reduction test avoids the use of radioisotopes and costly material and equipment; it is reliable, reproducible, and convenient for rapid routine susceptibility testing of MAC.**

Evaluation of bacteriostatic and bactericidal activities is essential for the assessment of susceptibility to antimycobacterial drugs before treatment and for screening and development of new drugs. In this study, we selected *Mycobacterium avium-M. intracellulare* complex (MAC) to evaluate a new method of determining bacterial growth and the MICs of clofazimine, resorcinomycin A, and the quinolone PD 127391 against MAC. MAC is an intracellular pathogen that causes a high rate of morbidity and mortality in AIDS patients (15).

When testing of MAC susceptibility to drugs is based on growth on solid media, at least 12 days of incubation are required (7). A rapid radiometric method (BACTEC) used recently to determine the MIC of ansamycin for MAC quantified $^{14}CO_2$ produced by mycobacteria growing in broth containing $^{14}$C-labeled palmitic acid (7). MICs of drugs for MAC also can be determined by visual inspection of bacterial growth in microplates; however, MIC data are obtained after 2 weeks of incubation (11).

A rapid, highly sensitive, quantitative, and less laborious technique that does not involve the use of radioactive materials or uncommon and expensive analytical equipment and materials is proposed in this study. The method is based on the ability of metabolically active cells to reduce dimethylthiazol-diphenyltetrazolium bromide (MTT) to formazan. Reduction of tetrazolium salts by mycobacteria and other microorganisms has been documented (12, 14).

In this study, we demonstrated that the MTT reduction assay was very sensitive to detection of MAC growth, enabling us to determine accurate MICs of clofazimine (a drug used for multiple-regimen therapy of MAC infections) (1, 9), resorcinomycin A (10), and PD 127391 (13) against MAC.

## MATERIALS AND METHODS

**Antimycobacterial agents and reagents.** Clofazimine was obtained from CIBA-GEIGY, Basel, Switzerland. A stock solution was prepared by dissolving 1 mg of clofazimine in 0.5 ml of dimethyl sulfoxide–0.5 ml of methanol–1.5 μl of 6 N HCl. Resorcinomycin A (10) was a gift from Taichiro Komeno of Shinogi Research Laboratories (Osaka, Japan). PD 127391, a new experimental quinolone (13), was provided by Parke-Davis Pharmaceutical Research Division (Ann Arbor, Mich.). Stock solutions of 250 μg of resorcinomycin A or PD 127391 per ml were prepared by dissolving 1 mg of the drugs in 100 μl of ethanol or methanol, respectively, and volumes were adjusted to 4 ml with saline. MTT, sodium dodecyl sulfate (SDS), and *N*,*N*-dimethylformamide (DMF) were obtained from Sigma Chemical Co. (St. Louis, Mo.). MTT and extraction buffer were prepared as previously described (5). In brief, MTT was dissolved at a concentration of 5 mg/ml in phosphate-buffered saline at room temperature. The solution was then sterilized by filtration and stored at 4°C in dark vials equipped with tight caps. It was prepared fresh every 15 days. Extraction buffer was prepared by dissolving 20% (wt/vol) SDS at 37°C in a solution of 50% each DMF and demineralized water; the pH was adjusted to 4.7.

**Culture and growth of mycobacteria.** MAC 101, obtained from Children's Hospital, Los Angeles, Calif., and isolates of *M. avium* (strain 35713) and *M. intracellulare* (strain 35761), obtained from the American Type Culture Collection (ATCC), were maintained on slants of Lowenstein-Jensen medium (Remel, Lenexa, Kans.). Culture suspensions were prepared by growing an initial inoculum on Middlebrook and Cohn 7H11 agar plates (BBL, Becton Dickinson, Cockeysville, Md.) and subculturing in Middlebrook 7H9 broth (MBB) (Difco Laboratories, Detroit, Mich.) containing 0.5% glycerol (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and 10% OADC (oleic acid-albumin-dextrose-catalase) enrichment (Remel) for 72 h at 37°C. The culture suspensions were shaken and sonicated in a bath type ultrasonicator (output power, 80 W; Laboratory Supplies Co., Hicksville, N.Y.) until visible clumps were disrupted (usually 15 to 30 s). Next, cultures were diluted in the same broth to yield a concentration of 3 Klett units/ml by using a Klett-Summerson colorimeter (Klett Manufacturing, Brooklyn, NY). Suspensions of 3 Klett units were frozen at −70°C until used (cultures were stored no more than 1 month in a freezer, and fresh cultures were prepared monthly). The 3-Klett-unit culture suspensions were thawed, shaken, and sonicated for 90 s before use. This procedure yielded a suspension of actively growing culture containing $10^7$ viable bacilli per ml, as confirmed by plate counts on 7H11 agar.

**MTT reduction assay.** Ten microliters of the 5-mg/ml stock solution of MTT was added to each well in 96-well microtiter plates containing bacteria (Becton Dickinson Co., Lincoln Park, N.J.). After incubation periods ranging from 1 to 6 h at 37°C, 100 μl of extraction buffer was added to each well. After overnight incubation at 37°C, optical densities were measured at 570 nm by using a microplate reader (Molecular Devices Corporation, Palo Alto, Calif.). MBB (incubated with MTT and extraction buffer) was used as a blank for resorcinomycin A and PD 127391. For clofazimine (4), the absorbances obtained with the drug alone were subtracted from those obtained with treated bacilli.

**Effect of time on MTT reduction by MAC.** To determine the optimal period of incubation of MAC with MTT, 20-ml volumes of MBB were inoculated with $10^5$ bacilli per ml in 50-ml culture tubes (Becton Dickinson), which were then incubated at 37°C and 160 rpm in an incubator-shaker (New Brunswick Scientific, Edison, N.J.) for 2 to 8 days. After each time point, 100-μl aliquots were distributed in six wells on microtiter plates. MTT was added to the wells and plates were incubated from 1 to 6 h, after which extraction buffer was added and plates were incubated overnight. Absorbances were then read.

**Determination of MAC growth by MTT reduction test.** The analytical sensitivity of the MTT test in assessing MAC growth was evaluated by incubating serially diluted MAC suspension in microtiter plates for 2, 6, and 10 days in a

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humidified incubator at 37°C and 5% $CO_2$. At each time point, MTT was added to all wells for 3 h. After extraction buffer was added and plates were incubated overnight, absorbances were determined.

**Comparison of abilities of MTT test and [$^3$H]glycerol uptake assay to determine MAC growth.** The analytical sensitivities of the MTT reduction test and the [$^3$H]glycerol uptake assay to detect MAC growth in microtiter plates and in tubes were compared. MAC growth in microtiter plates was determined by adding an inoculum of $10^6$ bacilli per ml from a 3-Klett-unit MAC suspension to wells containing 100 μl of MBB. The cultures were then grown for 2, 4, 6, 8, and 10 days in a humidified incubator at 37°C and 5% $CO_2$. After each time point, the culture wells were incubated with MTT for 3 h. Next, extraction buffer was added and the plates were incubated overnight. Absorbances were measured as described above. For [$^3$H]glycerol uptake assays, the same inoculum of bacteria was added to 100 μl of MBB without added glycerol. A 20-μl aliquot of [$^3$H]glycerol (ICN Biochemicals, Irvine, Calif.) was added to each well at a concentration of 50 μCi/ml (2). The plates were incubated for 2, 4, 6, 8, and 10 days in a humidified incubator at 37°C and 5% $CO_2$. At the end of the incubation periods, 50 μl of 5.25% sodium hypochlorite was added to each well and bacteria were harvested onto fiberglass filters by using a semiautomatic cell harvester (Cambridge Technology, Watertown, Mass.). The filter paper discs were dried overnight in separate vials and analyzed for radioactivity incorporated into the bacteria by using Scintiverse II (Fisher Scientific, Fair Lawn, N.J.) and a scintillation counter (Packard Instrument Company, Laguna Hills, Calif.).

MAC growth in tubes was examined by inoculating 10 ml of MBB with $10^5$ bacilli per ml and incubating the suspension for 2, 4, 6, 8, and 10 days at 37°C in an incubator-shaker at 160 rpm. After this, the tubes were sonicated in a bath type sonicator for 15 s (to disrupt visible clumps) and their contents were homogenized with a vortex mixer. One milliliter of this suspension was mixed with 100 μl of MTT, and the mixture was incubated for 3 h. Next, 1 ml of extraction buffer was added and incubation was continued overnight. After this, 100-μl aliquots were distributed into each of six wells and absorbances were determined. For the [$^3$H]glycerol uptake technique, 10 ml of MBB without added glycerol was inoculated with $10^5$ bacilli per ml plus 1 ml of [$^3$H]glycerol (50 μCi/ml). The tubes were then incubated at 37°C for 2, 4, 6, 8, and 10 days in an incubator-shaker at 160 rpm. After each time point, 100-μl aliquots were distributed into each of six wells and then 50 μl of bleach per well was added and bacteria were harvested as explained above.

**MIC determination.** MICs were assessed by adding 20-μl aliquots of a 3-Klett-unit MAC suspension ($10^7$ bacilli per ml) to each well in microtiter plates containing the drugs diluted with 100 μl of MBB (four replicate samples). The plates were prepared by adding 100 μl of stock solutions (100 to 250 μg/ml) of the drugs to the first well and making serial twofold dilutions up to 11 wells with MBB. Well 12 in all rows was left drug free so that these wells served as controls for bacterial growth (11). The plates were always prepared fresh immediately before inoculation. After inoculation of bacteria, the plates were incubated for 2, 4, 6, and 8 days at 37°C and 5% $CO_2$ in a humidified atmosphere. The MTT reduction test was performed after each time point, as explained above.

The results obtained throughout this study were expressed as means ± standard deviations for at least four replicate samples from one representative experiment; all experiments were performed three times.

## RESULTS

**Optimal incubation time of MAC with MTT.** To determine an optimal incubation period of MAC with MTT dye, bacilli were cultured for 2, 4, 6, and 8 days and then incubated with MTT for 1 to 6 h. As shown in Fig. 1, absorbances resulting from MTT reduction were observed to increase in a manner proportional to days of culture. MTT reduction reached a plateau, however, at 3 h of incubation with the dye (Fig. 1, inset). Therefore, an incubation period of 3 h with MTT, regardless of the number of days of culture, was considered optimal for determining MAC growth by MTT uptake.

**Analytical sensitivity of MTT dye for detection of MAC growth.** The accuracy of the MTT dye in determining MAC growth was assessed by using 10-fold serial dilutions of MAC suspension (10 to $10^6$ bacilli per ml). As shown in Fig. 2, MTT reduction correlated with the number of bacteria used to initiate cultures. As shown in the inset, detectable absorbances were observed even with as few as 10 bacilli per ml.

**Comparison of abilities of MTT test and [$^3$H]glycerol uptake assay to determine MAC growth.** The analytical sensitivities of a radiometric ([$^3$H]glycerol uptake) assay and the MTT colorimetric method to detect MAC growth were compared. Microtiter plates and culture tubes under rotation were used to culture the bacilli. When culturing MAC in microtiter plates, we observed the MTT technique to be more sensitive than the radiometric method in assessing the growth of bacilli. The radiometric method was less sensitive to detecting MAC growth during the first 8 days of culture, whereas the colorimetric assay measured growth from day 2 of culture (Fig. 3).

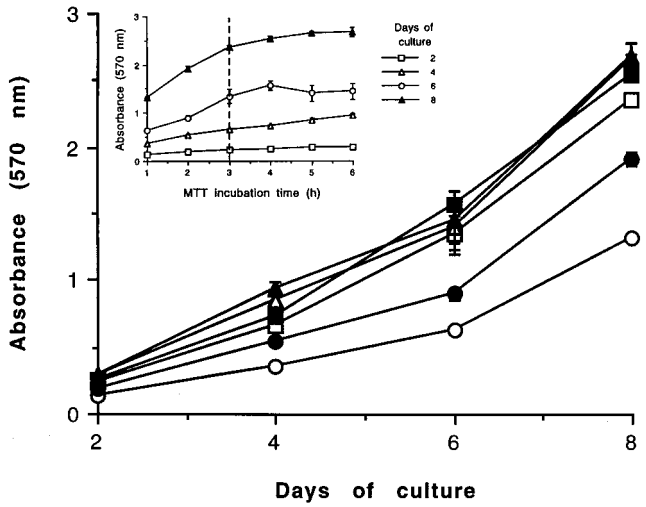


FIG. 1. Effect of incubation time on MTT reduction by MAC. An inoculum of $10^5$ bacilli per ml was grown in culture tubes under rotation. Aliquots were taken at days 2, 4, 6, and 8 and incubated with MTT for 1 to 6 h as explained in the text. Formazan was dissolved by adding extraction buffer. Absorbances were determined after overnight incubation. The inset shows the inverse plot emphasizing the effect of incubation time of bacilli with the MTT dye on formazan production (a plateau was reached at 3 h of incubation with MTT). The data represent the means ± standard deviations (error bars) for six replicate samples. Symbols for main diagram: ○, incubation time of 1 h; ●, 2 h; □, 3 h; ■, 4 h; △, 5 h; ▲, 6 h.

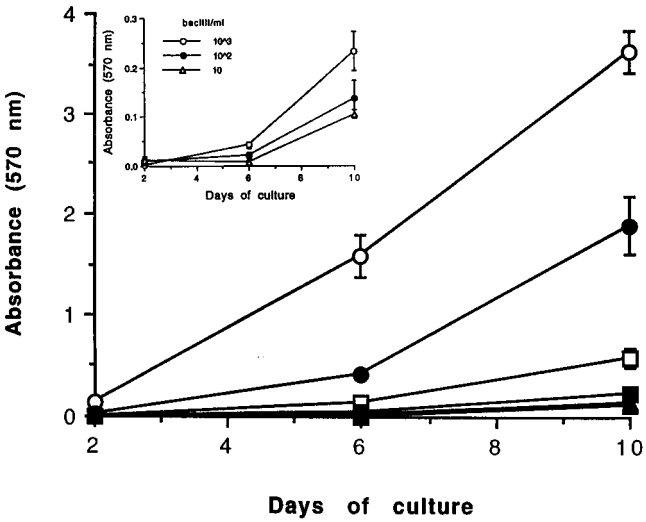


FIG. 2. Effect of initial inoculum size on MTT reduction by MAC. Microtiter wells were seeded with 10-fold dilutions of MAC suspension (10 to $10^6$ bacilli per ml) and incubated for 2, 6, and 10 days. MTT was added at each time point for 3 h, and formazan was dissolved by adding extraction buffer. Absorbances were determined after overnight incubation. The inset shows an expanded plot showing the absorbances obtained with low inocula of mycobacteria (10 to $10^3$ bacilli per ml). The data represent the means ± standard deviations (error bars) for four replicate samples. Symbols for main diagram: ○, $10^6$ bacilli per ml; ●, $10^5$; □, $10^4$; ■, $10^3$; △, $10^2$; ▲, 10.

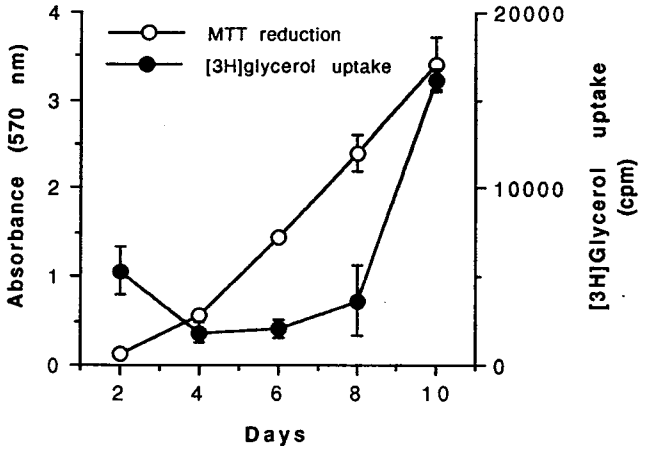


FIG. 3. Determination of MAC growth by MTT test and [$^3$H]glycerol uptake in microtiter plates. The capacities of the MTT reduction test and [$^3$H]glycerol uptake assay to assess MAC growth in microtiter plates were compared. An inoculum of $10^6$ bacilli per ml was grown in plates for 2 to 10 days. MTT was added at each time point for 3 h, and formazan was dissolved by adding extraction buffer. Absorbances were determined after overnight incubation. For [$^3$H]glycerol uptake experiments, bacteria were harvested after each time point and radioactivity incorporated within bacilli was determined as explained in the text. The data represent the means ± standard deviations (error bars) for four replicate samples.

When the bacilli were cultured in tubes, however, the two methods showed comparable analytical sensitivities (Fig. 4).

**MIC determination.** We preferred using microtiter plates to determine MICs because in comparison with tubes they save time and material. The MIC was determined as the lowest concentration of drug that significantly inhibited bacterial growth (as determined by a decrease in absorbances) over 4 to 8 days of treatment, did not allow resumption of growth, and caused at least 99.6% MAC growth inhibition by day 8 (absorbances from untreated bacilli were taken as a control for MAC growth; percent reductions of absorbances for treated bacteria compared with the control were expressed as MAC growth inhibition). As the data in Tables 1, 2, and 3 demonstrate, the MICs of clofazimine, resorcinomycin A, and PD 127391 against MAC were 3, 15, and 31 μg/ml, respectively. We found the MICs of clofazimine against *M. avium* (strain 35713) and *M. intracellulare* (strain 35761) to be 1.5 and 3 μg/ml, respectively (Tables 4 and 5). Solvents used to prepare the drugs had no effect on mycobacterial growth (data not shown).

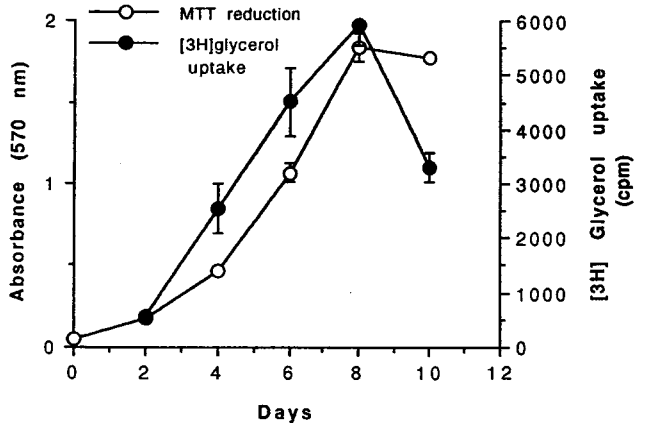


FIG. 4. Determination of MAC growth by MTT test and [$^3$H]glycerol uptake in culture tubes. The capacities of the MTT reduction test and [$^3$H]glycerol uptake assay to assess MAC growth in culture tubes were compared. An inoculum of $10^5$ bacilli per ml was grown in tubes for 2 to 10 days with rotation, as explained in the text. Aliquots were then transferred to microtiter plates at each time point, and MTT was added for 3 h. Formazan was dissolved by adding extraction buffer, and absorbances were determined after overnight incubation. For [$^3$H]glycerol uptake experiments, aliquots were similarly transferred to plates at each time point and bacteria were harvested. Radioactivity incorporated in bacilli was then determined as explained in the text. The data represent the means ± standard deviations (error bars) for six replicate samples.

TABLE 1. Determination of MIC of clofazimine against MAC 101

| Clofazimine concn (μg/ml) | $A_{570}$[a] at day: | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 0 | 0.431 | 0.837 | 1.406 | 2.435 |
| 0.037 | 0.184 | 0.539 | 1.146 | 1.988 |
| 0.075 | 0.149 | 0.443 | 0.859 | 1.772 |
| 0.15 | 0.116 | 0.286 | 0.521 | 1.339 |
| 0.37 | 0.087 | 0.118 | 0.137 | 0.294 |
| 0.75 | 0.060 | 0.054 | 0.053 | 0.071 |
| 1.5 | 0.055 (87)[b] | 0.039 (95) | 0.027 (98) | 0.066 (97) |
| 3 | 0.039 (91) | 0.016 (98) | 0.006 (99.6) | 0.004 (99.8) |
| 6 | 0.019 | 0 | 0 | 0 |
| 12 | 0.007 | 0 | 0 | 0 |
| 25 | 0.005 | 0 | 0 | 0 |
| 50 | 0.003 | 0 | 0 | 0 |

[a] Standard deviations from the absorbances shown are less than 5%.
[b] Values in parentheses correspond to MAC growth inhibition (percentage of control value at each time point).

## DISCUSSION

Susceptibility of mycobacteria to drugs is usually tested by determining the MIC (6). Susceptibility testing of MAC has not been standarized, although a radiometric method (BACTEC) has been used in some laboratories (7, 8). The BACTEC method requires less time and labor than do conventional methods (sampling and plating [7] or determining the MIC in 7H10 agar medium [3]). However, it requires the use of radioactive materials and costly analytical equipment. Determining MICs by visual inspection gives a good estimation of MAC susceptibility to drugs. It usually takes 2 weeks, however, to obtain confirmatory data (11).

TABLE 2. Determination of MIC of resorcinomycin A against MAC 101

| Resorcinomycin A concn (μg/ml) | $A_{570}$[a] at day: | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 0 | 0.098 | 0.410 | 1.084 | 1.819 |
| 0.075 | 0.072 | 0.317 | 0.740 | 1.312 |
| 0.15 | 0.052 | 0.241 | 0.527 | 0.947 |
| 0.37 | 0.041 | 0.165 | 0.327 | 0.490 |
| 0.75 | 0.038 | 0.107 | 0.187 | 0.193 |
| 1.5 | 0.027 | 0.079 | 0.122 | 0.101 |
| 3.7 | 0.022 | 0.071 | 0.109 | 0.092 |
| 7 | 0.013 (86.7)[b] | 0.029 (92.9) | 0.049 (95.5) | 0.049 (97) |
| 15 | 0.010 (89.8) | 0.014 (96.6) | 0.012 (98.9) | 0.007 (99.6) |
| 31 | 0.004 | 0.008 | 0.004 | 0 |
| 62 | 0.004 | 0.006 | 0.004 | 0.003 |
| 125 | 0.003 | 0.005 | 0.003 | 0.003 |

[a] Standard deviations from the absorbances shown are less than 5%.
[b] Values in parentheses correspond to MAC growth inhibition (percentage of control value at each time point).

TABLE 3. Determination of MIC of PD 127391 against MAC 101

| PD 127391 concn (μg/ml) | $A_{570}$[a] at day: | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 0 | 0.102 | 0.416 | 1.076 | 1.709 |
| 0.075 | 0.047 | 0.106 | 0.152 | 0.154 |
| 0.15 | 0.035 | 0.065 | 0.096 | 0.070 |
| 0.37 | 0.031 | 0.046 | 0.067 | 0.040 |
| 0.75 | 0.024 | 0.038 | 0.048 | 0.032 |
| 1.5 | 0.020 | 0.035 | 0.042 | 0.027 |
| 3.7 | 0.019 | 0.027 | 0.034 | 0.024 |
| 7 | 0.014 | 0.024 | 0.034 | 0.017 |
| 15 | 0.013 (87)[b] | 0.017 (95.9) | 0.024 (97.8) | 0.016 (99) |
| 31 | 0.008 (92) | 0.006 (98.6) | 0.003 (99.7) | 0 (100) |
| 62 | 0.006 | 0.002 | 0.001 | 0 |
| 125 | 0 | 0.001 | 0 | 0 |

[a] Standard deviations from the absorbances shown are less than 5%.
[b] Values in parentheses correspond to MAC growth inhibition (percentage of control value at each time point).

TABLE 5. Determination of MIC of clofazimine against *M. intracellulare*[a]

| Clofazimine concn (μg/ml) | $A_{570}$[b] at day: | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 0 | 0.221 | 0.909 | 2.945 | 3.380 |
| 0.037 | 0.179 | 0.543 | 1.928 | 3.390 |
| 0.075 | 0.170 | 0.418 | 1.159 | 2.307 |
| 0.15 | 0.157 | 0.325 | 0.619 | 1.686 |
| 0.37 | 0.151 | 0.154 | 0.222 | 0.393 |
| 0.75 | 0.105 | 0.078 | 0.062 | 0.094 |
| 1.5 | 0.103 (53)[c] | 0.037 (95.9) | 0.020 (99.3) | 0.030 (99.1) |
| 3 | 0.060 (72.8) | 0.012 (98.7) | 0.007 (99.8) | 0 (100) |
| 6 | 0.040 | 0 | 0 | 0 |
| 12 | 0.016 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 |

[a] ATCC 35761.
[b] Standard deviations from the absorbances shown are less than 5%.
[c] Values in parentheses correspond to MAC growth inhibition (percentage of control value at each time point).

Our study demonstrated that MICs can be determined by measuring MAC growth for 8 days by the MTT reduction assay. The MTT reduction test (the optimal incubation period of MAC with MTT was 3 h; longer periods did not increase formazan production significantly [Fig. 1]) could detect the progression of growth of different sizes of inoculum (10 to $10^6$ bacilli per ml) with a high degree of analytical sensitivity, as shown in Fig. 2. When we compared this technique with a conventional method (measuring [$^3$H]glycerol uptake) (2) to determine MAC growth, we observed that the MTT test was able to monitor growth whether the bacteria were grown in microtiter plates or culture tubes (Fig. 3 and 4). The radiometric technique was less sensitive to detection of MAC growth during the first 8 days of culture in microtiter plates (Fig. 3); this was probably because the method is limited to detecting high numbers of growing bacteria. However, when MAC was cultured in tubes under rotation (which facilitates MAC growth), the analytical sensitivities of the radiometric and colorimetric techniques were comparable (Fig. 4); reduced absorbances (formazan production) or counts per minute ([$^3$H]glycerol uptake), observed after 8 days of culture, may be caused by a decline in the number of viable organisms cultured in tubes, as reported by others (7), or they may occur because the stationary phase has been reached (Fig. 4).

TABLE 4. Determination of MIC of clofazimine against *M. avium*[a]

| Clofazimine concn (μg/ml) | $A_{570}$[b] at day: | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| 0 | 0.460 | 1.317 | 2.700 | 3.232 |
| 0.037 | 0.393 | 0.887 | 1.606 | 2.049 |
| 0.075 | 0.207 | 0.665 | 1.208 | 2.122 |
| 0.15 | 0.140 | 0.403 | 0.347 | 1.301 |
| 0.37 | 0.088 | 0.261 | 0.140 | 0.353 |
| 0.75 | 0.087 (81)[c] | 0.082 (93.7) | 0.040 (98.5) | 0.096 (97) |
| 1.5 | 0.049 (89) | 0.054 (95.9) | 0.021 (99) | 0.011 (99.7) |
| 3 | 0.041 | 0.005 | 0 | 0 |
| 6 | 0.022 | 0 | 0 | 0 |
| 12 | 0.017 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 |

[a] ATCC 35713.
[b] Standard deviations from the absorbances shown are less than 5%.
[c] Values in parentheses correspond to MAC growth inhibition (percentage of control value at each time point).

The ability of the MTT test to assess mycobacterial growth, as demonstrated in this study, enabled us to determine the MICs of drugs against MAC (Tables 1, 2, and 3). The reported MICs of clofazimine and resorcinomycin A (1 and 8 μg/ml, respectively) were determined after 2 weeks of culture by visual inspection (11); the MICs of these drugs reported in the present study (3 and 15 μg/ml, respectively) were obtained at 8 days of culture by the MTT reduction test. The MTT test's analytical sensitivity, however, resulted in slightly higher MICs of these drugs. We also determined the MICs of clofazimine against *M. avium* (strain 35713) and *M. intracellulare* (strain 35761) by this technique (Tables 4 and 5). Thus, determination of the MICs of drugs by the MTT method could be used for other species of mycobacteria as well. The MTT method was found to be faster and more accurate in determining the effect of drugs on MAC growth than visual inspection was.

In conclusion, we support the MTT assay as a rapid, highly sensitive, and quantitative colorimetric microassay for susceptibility testing of MAC. It does not require the use of radioisotopes or costly materials and equipment. It is based on the reduction of MTT to formazan by metabolically active cells, and allows accurate and reproducible determination of MICs of drugs against MAC. We believe that this is a promising technique for rapid and convenient routine susceptibility testing of MAC and other species of mycobacteria.

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