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Improved Agar Gradient-Plate Technique

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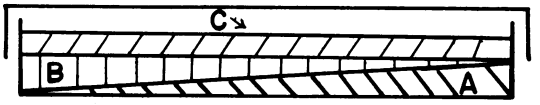
The gradient-plate technique (2) has been used for various microbiological studies including the determination of minimal inhibitory concentrations (MIC) of antimicrobial compounds (1). To use the gradient-plate method for determining the MIC of antimicrobial compounds, it is essential to have uniform microbial growth on the agar medium; otherwise, it is difficult to determine a sharply defined area of growth inhibition. The conventional agar gradient-plate method has been found to be unsatisfactory for determining the MIC of chemotherapeutic compounds or antibiotics which inhibit filamentous fungi because the latter frequently do not grow uniformly when streaked on the surface of agar plates. This problem can be circumvented somewhat by seeding the top layer of conventional agar gradient-plates with the test organism; however, the nonuniform depth of the seeded agar layer precludes the determination of a sharply defined area of fungal inhibition.



A. agar medium plus amphotericin B (25 µg/ml)

B. agar medium

C. agar medium seeded with test organism

FIG. 1. *Diagrammatic view of a trilevel gradient-agar plate.*

A modification of the original gradient-plate method has been devised and found to improve the utility and accuracy of this technique when used to determine the MIC of antifungal compounds. Trilevel gradient-agar plates were prepared according to the diagram in Fig. 1, with the antifungal compound incorporated into the bottom layer. A saline (0.85% NaCl) suspension of spores and mycelia from a washed slant culture of *Aspergillus niger* (ATCC 1004) was incorporated into the thin top layer of agar. The volume of this layer of agar was 5 ml. For comparative purposes, conventional gradient-agar plates were prepared according to the original method of Szybalski (2). Sabouraud dextrose agar (Difco) and amphotericin B (E. R. Squibb & Sons, New York, N.Y.) were used in all experiments. All plates were incubated at 25 C in an inverted position.

TABLE 1. *Determination of MIC of amphotericin B*[a]

Trial	Conventional gradient-plate method[b]	Trilevel gradient-plate method[c]
1	13.9	13.9
2	7.0	14.7
3	8.3	11.1
4	18.2	14.0
5	10.9	12.1

[a] Values are expressed as micrograms per milliliter. The test microorganism was *A. niger* (ATCC 1004).
[b] Average MIC for the five trials was 11.66 µg/ml; standard error was 4.51 µg/ml.
[c] Average MIC for the five trials was 13.16 µg/ml; standard error was 1.50 µg/ml.

The results of representative experiments are summarized in Table 1. In contrast to the conventional gradient-plate technique, the trilevel plate method yielded a narrow range of MIC values, the average of which differed little within the individual trials. The obvious disadvantage of the trilevel agar plate method is that only one fungal strain or species can be tested per plate. However, this method reduces the number of experiments needed to obtain consistent MIC values.

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