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Correction for Bacterial Loss in In Vitro Dilution Models

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A new method to correct for bacteria lost to dilution in in vitro kinetic models is presented which includes the influence of the stationary bacterial growth phase. The method imposes an upper limit on bacterial density, in contrast to previous methods.

Several in vitro kinetic models which simulate in vivo drug concentration-time profiles use first-order dilution to decrease drug concentrations with respect to time (3, 4). Concurrent dilution or loss of bacteria along with the drug has been ignored (3), or observed bacterial densities have been corrected for loss due to dilution by making certain assumptions (4). In this report we identify situations in which existing correction factors can give unrealistic or inaccurate results and suggest an alternative correction factor.

A general diagram of common in vitro kinetic dilution models is presented in Fig. 1. Drug-free growth medium is pumped from flask A to flask B at a constant rate. Flask B contains bacteria and drug in bacterial growth medium; it is tightly sealed, thereby maintaining constant volume as medium flows through it. Medium, drug, and bacteria flow out of flask B at a constant rate, resulting in dilution of the drug and bacteria in the flask. Exposure of bacteria to drug regimens with different elimination half-lives are accomplished by changing the flow rate. Previous correction factors have accounted for bacteria lost from flask B due to flow by comparing the rate of change in bacterial density versus time for flowing and nonflowing conditions. Equations 1 and 2 give the rates of change in bacterial densities with (N) and without (N') bacterial loss.

$$\frac{dN}{dt} = (k_g - k_d - k_e)N \text{ (observed)} \quad (1)$$

$$\frac{dN'}{dt} = (k_g - k_d)N' \text{ (theoretical)} \quad (2)$$

where k_g, k_d, and k_e are rate constants for bacterial growth, bacterial death, and drug elimination, respectively. The difference between the solutions to equations 1 and 2 gives the bacterial density corrected for loss due to dilution as a function of time (t) (4).

$$N_t' = N_t e^{k_e t} \quad (3)$$

Use of this correction factor assumes that (i) removal of bacteria does not influence the growth kinetics of the remaining bacteria, (ii) bacterial growth kinetics are first-order, and (iii) the difference in the growth rate constants between the flowing and nonflowing conditions is a function only of the medium flow rate (2). All three assumptions become invalid when bacterial growth approaches the stationary phase. Violation of these assumptions overestimates the number of bacteria theoretically expected had there been no flow. The degree of overestimation is a function of flow and will therefore differ as a function of drug half-life. Comparisons of antibacterial effects for exposure conditions with different drug half-lives may therefore lead to incorrect conclusions due to this artifact.

Artificially large, corrected bacterial densities can be avoided by modifying equations 1 and 2 to include the limitation of maximum bacterial density (N_{max}) (1):

$$\frac{dN}{dt} = \frac{(k_g - k_d - k_e)N(N_{max} - N)}{N_{max}} \text{ (observed)} \quad (4)$$

$$\frac{dN'}{dt} = \frac{(k_g - k_d)N'(N_{max} - N')}{N_{max}} \text{ (theoretical)} \quad (5)$$

where N_{max} is the average bacterial density in the stationary phase observed in flask B under nonflowing conditions. The difference between the solutions to equations 4 and 5 gives the bacterial density corrected for loss due to dilution.

$$N_t' = \frac{(N_{max})N_t}{N_t + (N_{max} - N_t)e^{-k_e t}} \quad (6)$$

The correction does not require that k_g and k_d remain constant.

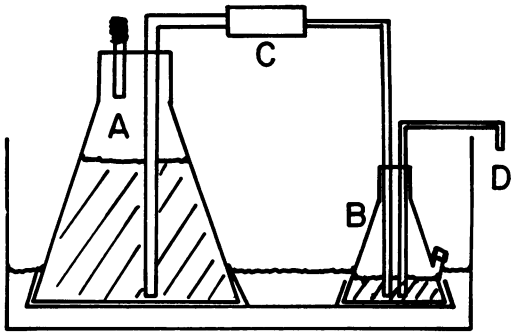

FIG. 1. Schematic representation of in vitro dilution models. Medium is transferred from the reservoir flask (A) to the test flask (B) by the pump (C). Flask B maintains a constant volume; bacteria, drug, and medium are sent to waste (D).

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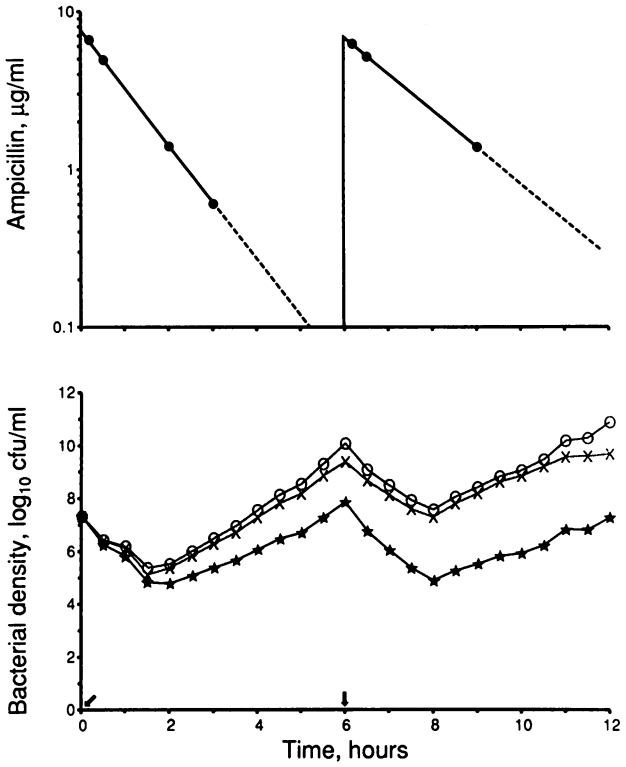

FIG. 2. Time course of *E. coli* ATCC 12407 concentration when exposed to ampicillin in an in-vitro dilution model as shown in Fig. 1. Symbols: ★, actual bacterial concentration; ○, concentration corrected to the no-flow case by equation 3; X, concentration corrected to the no-flow case by equation 6.

Corrected bacterial densities will be similar when calculated by equation 3 or 6 until N_{max} is approached. The difference between the previous flow correction (equation 3) and the new correction (equation 6) when applied to actual bacterial densities is shown in Fig. 2. The data shown were obtained from a study in which *Escherichia coli* ATCC 12407 (10^7 CFU/ml) were incubated with ampicillin (peak concentration, 6 × MIC) in an in vitro model (Fig. 1) with a flow rate which produced an ampicillin half-life of 1 h. Bacterial densities were quantitated by a viable-cell assay. Ampicillin concentrations (Fig. 2) were quantitated by microassay (5).

The N_{max} value was determined to be $10^{9.5}$ in a nonflowing system without antibiotics. The corrected bacterial concentrations are similar for concentrations less than N_{max} but diverge as N_{max} is approached.

In conclusion, equation 3 provides correct results when bacterial density is less than the stationary-phase density. However, equation 6 provides a more realistic result when the stationary-phase density is approached.

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