# Turbidimetric Determination of Blood Aminoglycoside Levels by Growth Curve Analysis

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**Abstract** □ A procedure to assay rapidly blood aminoglycoside levels was developed based on growth curve analysis of continuous absorbance measurements of a bacterial test strain. Of the means available for monitoring bacterial growth quantitatively, turbidimetric measurement was selected because it can be used to perform a clinical assay in <4 hr. Continuous turbidimetric measurements provide information as soon as the antimicrobial agent affects growth, which often occurs within 60 min of the start of an assay. Several bacterial isolates were evaluated before *Staphylococcus aureus* (MHMC 386) was chosen as the test microorganism. This isolate was quite sensitive to the aminoglycoside antibiotics, permitted the rapid measurement of blood levels of amikacin, gentamicin, kanamycin, and tobramycin, and exhibited a linear dose–response relationship of turbidity over a wide range of antibiotic concentrations.

**Keyphrases** □ Antibiotics, aminoglycoside—amikacin, gentamicin, kanamycin, and tobramycin, effect on bacterial growth, turbidimetric measurement of blood levels □ Antibacterials—amikacin, gentamicin, kanamycin, and tobramycin, effect on bacterial growth, turbidimetric measurement of blood levels □ Spectrophotometry—turbidimetric determination of blood levels of amikacin, gentamicin, kanamycin, and tobramycin, effect on bacterial growth

The concentration at which aminoglycoside antibiotics injure animal cells is only two to four times greater than the concentration required to inhibit most common bacterial pathogens. In addition, distribution of the drug to the various body compartments is not the same in all individuals. Accordingly, a rapid assay is required for the optimal usage of aminoglycosides.

## BACKGROUND

There is a direct relationship between bacterial growth measured turbidimetrically and the increase in bacterial numbers, collective mass, and cell volume. During the period when cells are dividing with a constant doubling time, the number of cells increases exponentially. If $t_0$ is the starting time and $t_2$ is the time required for the average bacterium to become two, then the number of bacteria ($N_2$) at $t_2$ can be calculated from:

$$\log N_2 = N_0\, 10^{t_0/t_2} \qquad \text{(Eq. 1)}$$

where $N_0$ is the number of bacteria at the starting time. The Rayleigh–Debye theory can be used to interrelate turbidity and cell mass if turbidity can be measured as a function of the ratio of the particle size to the wavelength at maximum turbidity and if the refractive indexes of the solvent and the particle are not significantly different. The concentration of bacteria per unit volume of suspending medium can be calculated from:

$$NVD = \frac{R_\theta 3\lambda D \sin^3(\theta/2)}{(\Delta\mu)(M-1)\mu x} \qquad \text{(Eq. 2)}$$

where $\theta$ is the modification of the light intensity due to the finite size of the particle, $V$ is the volume of the particle, $N$ is the number of particles per milliliter, $R_\theta$ is the reduced intensity for unpolarized light scattered at an angle $\theta$ from an incident beam, $D$ is the density of the particle, $\lambda$ is the wavelength of the light, $M$ is the ratio of the refractive indexes of the particle and the medium, and $\mu$ is the refractive index function (1).

Equation 2 indicates that at 0°, light-scattering turbidity is related directly to the number or mass of bacteria (1–3). Based on this theory, spectrophotometric techniques were developed to assay antimicrobial agents. These systems have been used primarily in industry, where relatively high concentrations of antibiotics are measured. The development of clinical turbidimetric assays has suffered from a lack of sensitive equipment and the labor-intensive nature of this assay.

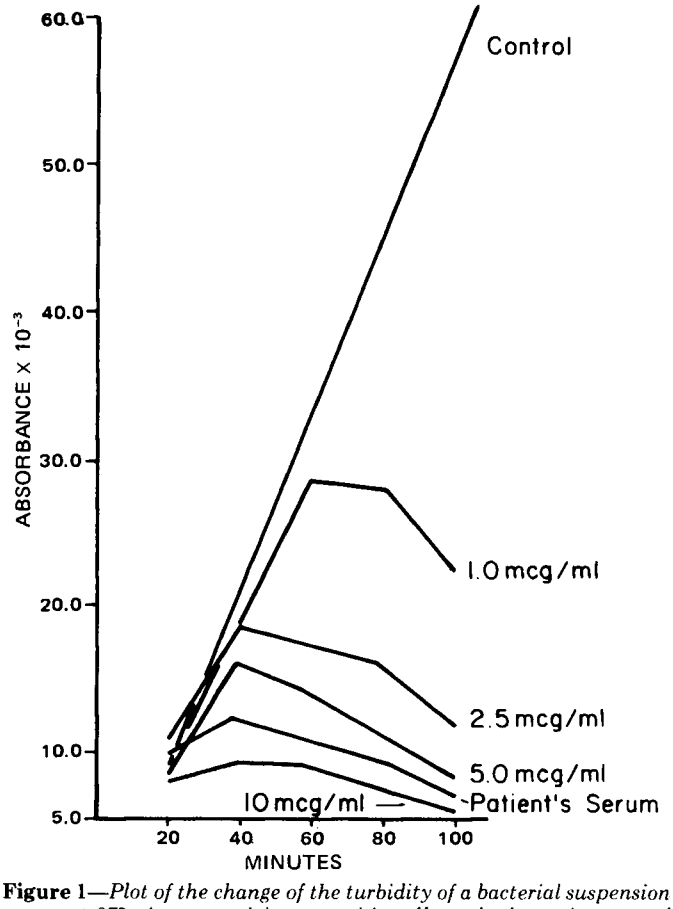


**Figure 1**—*Plot of the change of the turbidity of a bacterial suspension grown at 37°. As gentamicin exerted its effect, the bacteria stopped growing and the absorbance decreased.*

## EXPERIMENTAL

A sensitive, low-noise spectrophotometer[1] was developed recently that automatically determines the bacterial density every 5 min under temperature-controlled conditions (4). This instrument currently is sold for antibiotic susceptibility tests. Because of its availability and ability to yield continuous printouts of absorbance measurements without manipulation of the cell, it seemed a likely candidate for the clinical assay of aminoglycosides.

A unique feature of this instrument is the design of the cell. The cell consists of two physically separated compartments: an upper compartment, which accepts the bacterial test strain and allows growth to occur to the log phase; and a lower compartment, which is divided into discrete, 1-cm³ chambers, with each chamber able to receive one antibiotic test specimen. A technique to determine the concentration of blood amino-

[1] MS-2, Abbott Laboratories, Dallas, Tex.



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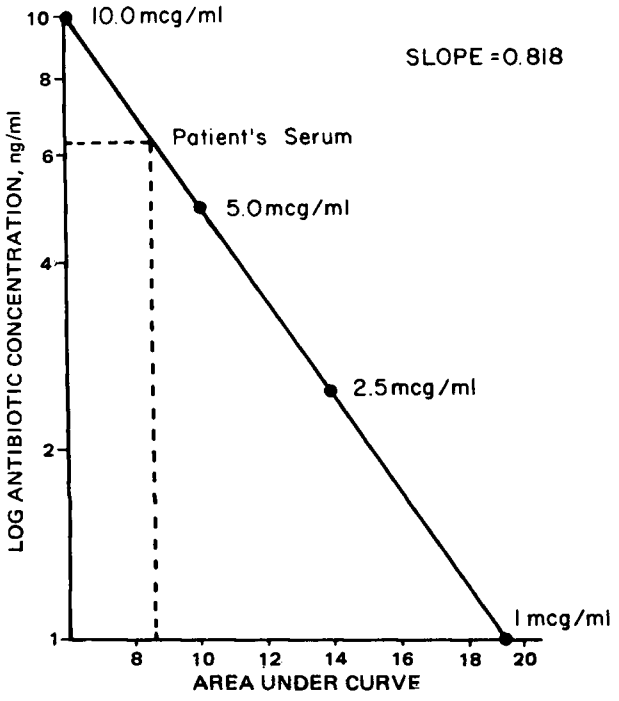


**Figure 2**—*Plot of area under the curve produced from a graph of absorbance* versus *time (Fig. 1) calculated for each antibiotic concentration, the control (no antibiotic), and the patient's serum. The area under the curve was proportional to the antibiotic concentration in the patient's serum. The amount of antibiotic in a patient's serum can be calculated by direct interpolation from a plot of log antibiotic concentration* versus *area under the curve. The patient's serum concentration was 6.8 μg/ml.*

glycoside levels using growth curve analysis from turbidimetric measurements supplied by this instrument was developed.

The selected strain of *Staphylococcus aureus* (MHMC 386) was sensitive to the aminoglycoside antibiotics and did not clump when grown in broth. *S. aureus* MHMC 386 was resistant to penicillin and sensitive to amikacin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, oxacillin, tetracycline, tobramycin, and vancomycin. Antibiotic medium No. 5[2] was prepared according to directions from the manufacturer and adjusted to pH 7.4. A 17-ml volume of this medium was added to a sterile test tube. All antibiotics were obtained in powder form of known potency[3]. Aminoglycosides were prepared in standard solutions of 100 μg/ml in 0.1 *M* phosphate buffer (pH 7.9) (5). Stock solutions were frozen at −20° and stored for no longer than 90 days.

Human serum was obtained from normal blood bank donors and was heated to 56° for 30 min and filter sterilized (porosity 0.45 μm). For the measurement of serum antibiotic concentrations, the following dilutions in serum at pH 7.4 were made: amikacin, 25, 15, 10, and 5 μg/ml; gentamicin, 10, 5, 2.5, and 1 μg/ml; kanamycin, 25, 15, 10, and 5 μg/ml; and tobramycin, 10, 5, 2.5, and 1 μg/ml.

Forceps sterilized by alcohol and flaming were used to place filter paper disks[4] on a sterile petri plate, and 25 μl of each standard was added to individual disks. Twenty-five microliters of the patient's serum was added to each of two other disks. If the patient was receiving a β-lactam antibiotic, 0.25 ml of a broad-spectrum β-lactamase was added to 2 ml of the patient's serum prior to addition to the disks, and an appropriate correlation computation was made for this dilution. Each disk was placed in a separate compartment in the multichambered cell. Although the disks were used within 2 hr of preparation, the stability of aminoglycosides on the disks for long periods is not known.

[2] Difco Laboratories, Detroit, Mich.
[3] Ames Laboratories, Elkhardt, Ind.
[4] No. 740E, Schlicher and Schuell, Keane, N.H.

**Table I—Comparison of Blood Antibiotic Levels by Bioassay and Turbidity**

| Antibiotic | Number of Specimens | Discrepancy between Methods[a] 0–5% | 5–10% | 10–15% | 15–25% | >25% |
|---|---|---|---|---|---|---|
| Gentamicin | 112 | 20 | 38 | 28 | 13 | 2 |
| Kanamycin | 22 | 27 | 27 | 32 | 14 | 0 |
| Tobramycin | 26 | 15 | 42 | 36 | 3 | 4 |
| Amikacin | 11 | 9 | 45 | 36 | 9 | 0 |

[a] Percentages are rounded to the nearest whole number.

A suspension of *S. aureus* at 0.5 MacFarland's turbidity was made in 0.9% NaCl, and 0.1 ml of this suspension was added to 17 ml of antibiotic broth No. 5. Fifteen milliliters was added to the upper growth chamber of the cell, and the cell was placed in the spectrophotometer, which was temperature controlled at 35°. The bacterial suspension was physically separated from the antibiotics and the patient's serum until log-phase growth was entered as monitored by continuous turbidimetric reading. Utilizing a suction device built into the instrument, the bacterial suspension was drawn into compartments containing the disks. Incubation and continuous readings at 470 nm continued until the compartment with the smallest concentration of standard antibiotic had exerted its effect.

## RESULTS AND DISCUSSION

Figure 1 represents a typical determination. The area under the curve for each standard and the area under the curve of the patient's specimen were calculated by the least-squares method. The areas under the curve for the two individual patient serum compartments were averaged. Log antibiotic concentration was plotted *versus* the area under the curve (Fig. 2), and the amount of antibiotic in the patient's specimen was determined by direct interpolation from this graph.

Table I presents a comparison of results obtained by a standard bioassay method (5, 6) with those obtained by the turbidimetric assay. The bioassay and turbidimetric assays agreed, on the average, within 10% for the 161 blood specimens tested. The turbidimetric assay could determine 10 μg of gentamicin/ml within 160 min of the start of an assay, a period comparable to the radioimmunoassay method. As little as 1 μg of gentamicin/ml could be determined within 4 hr. The ability to determine physiological concentrations of the other aminoglycoside antibiotics was equally rapid.

## CONCLUSION

Under carefully controlled conditions, turbidimetric assay of antibiotics can be applied to clinical specimens. Because turbidimetric assays are amenable to automation and can be monitored continuously without human intervention, the data can be produced and interpreted with almost no expenditure of the technician's time. Currently, blood antibiotic level measurements are generally restricted to large medical institutions. With automated spectrophotometric systems, all medical facilities could determine blood antibiotic concentrations rapidly when potentially toxic antimicrobial agents are administered.

## REFERENCES

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