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This Week's Citation Classic

Bennett J V, Brodie J L, Benner E J & Kirby W M M. Simplified, accurate method for antibiotic assay of clinical specimens. *Appl. Microbiol.* 14:170-7, 1966. [Division of Infectious Diseases, Department of Medicine, University of Washington School of Medicine, Seattle, WA]

A specially designed agar punch was used to create multiple small agar wells in specified patterns on a large glass plate containing agar seeded with susceptible test organisms. Concentrations were determined by a procedure that exploited the natural curvilinear relationship between zone sizes around the filled wells and antibiotic concentrations. Small volumes of serum could then be tested directly and accurately over a wide range of clinically relevant concentrations without dilutions of the samples. [The *SCI*® indicates that this paper has been cited in more than 840 publications, making it the most-cited paper published in this journal.]

When Nature Prefers a Curve

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This work was accomplished with the superb technical assistance of Jean L Brodie while E. Jack Benner and I were fellows in infectious diseases with W.M.M. Kirby at the University of Washington in 1964.

Work began after the simple (and uncontested) declaration of our mentor that we needed a better bioassay (comparison of a test sample against a standard preparation) system for antibiotics. The assay in use at that time provided highly variable results and posed a variety of methodologic problems that are detailed in the paper but are too numerous to be fully recounted here.

One major problem derived from the well-recognized curvilinear relationship between the concentration of antibiotic standards and the sizes of the zones of inhibition they produced with sensitive test organisms in assay systems. Standard solutions were kept to a narrow range of known concentrations in an attempt to locate a portion of the dose-response curve that was most closely linear. Serum samples from patients were tested in several dilutions in the hope of finding a dilution within the range of the standards. Mathematical transformations of the inhibitory zone sizes had sometimes been used to straighten the line, but unmistakable traces of the underlying curve always remained even over narrow ranges.

We decided to accept rather than resist nature's message. We set up assay systems that used doubling concentrations of standards over an expanded range in order to encompass the clinically useful serum levels expected with each drug. We simplified the mathematics needed to determine the best-fitting curve and showed how the concentration of an unknown could be mathematically derived without the actual use of graph paper (a process that could now be done instantly with a computer spreadsheet!). With the assistance of a first-generation electronic calculator, we evaluated the accuracy of results based on the curvilinear relationship. The new procedure was tested on many quality control "unknowns" prepared within and outside of our laboratory, and it performed with remarkable accuracy and precision.

The procedure has been referred to as "the agar well diffusion method"[1] and "the large plate agar diffusion method."[2] It has been used exactly as described, as well as with various modifications, such as using paper disks on the agar surface instead of agar wells.[3] When the method has been checked,[2,4,5] it has performed accurately and precisely. It has been used for pharmacokinetic studies in serum and extravascular tissues of a wide variety of antimicrobials in both human and veterinary medicine. Half of the 24 publications citing the method in 1990 had authors who had also cited the paper in 1981, suggesting continued usefulness to them for nearly a decade. Further, 29 authors cited the method in more than one paper in a single year.

The bioassay method appears to have been cited frequently because it has repeatedly met the need to accurately and simply assay clinical samples in small volumes over wide ranges of concentrations. In short, the paper has delivered exactly as promised in its title—a characteristically direct and precise title that I fondly and gratefully recall was suggested by Kirby, who devoted many hours to ensure this paper was understandable.

1. **Thadepalli H, Bach V T, Mandal A K & Rambhatla K.** Is penicillin alone effective in enterococcal endocarditis—an experimental study in rabbits. *Chemotherapy* 27:340-9, 1981.
2. **Luthy R, Blaser J, Bonetti A, Siegenthaler W, Simmen H & Wise R.** Comparative multiple-dose pharmacokinetics of cefotaxime. moxalactam and ceflazidime. *Antimicrob. Agents Chemother.* 20:567-75. 1981.
3. **Thompson M I B, Atkinthor E, Matsen J M & Russo M** E. Piperacillin pharmacokinetics in subjects with chronic renal failure. *Antimicrob. Agents Chemother.* 19:450-3. 1981.
4. **Shapero R M. Matsen J M & Warwick W J.** Clindamycin therapy of staphylococcal pulmonary infections in patients with cystic fibrosis.*J Pediat.* 99:647-50, 1981.
5. **Wise R, Logan M, Cooper M, Ashby J P & Andrews J M.** Meropenem pharmacokinetics and penetration into an inflammatory exudate. *Antimicrob. Agents Chemother.* 34:1515-7, 1990.

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