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Editorial

Synergy, antagonism, and what the chequerboard puts between them

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The study of interactive effects between molecules has a long history. For antimicrobial drugs, the use of paired and triple combinations of inhibitory agents in the clinic often begins with tests *in vitro* that show positive interactions inhibiting the growth of target microorganisms. There are many models for experimental designs to measure such combination effects. One of the best known and very simple forms of such tests is the 'chequerboard' experiment in which a two-dimensional array of serial concentrations of test compounds is used as the basis for calculation of a fractional inhibitory concentration index (FICI) to demonstrate that paired combinations of agents can exert inhibitory effects that are more than the sum of their effects alone (synergy; FICI < 1.0), or to less than the sum of their effects alone (antagonism; FICI > 1.0).

The popularity of the FICI approach is undeniable. Scrutiny of issues in this journal and in *Antimicrobial Agents and Chemotherapy*, over the 5 year period January 1998–December 2002, revealed a total of 96 papers in which combinations of antimicrobial agents were tested *in vitro*. Among these, FICI determinations were used in 58 (60.4%): alone in 37, or in addition to other approaches in 21. Time–kill methodology was used in 35 papers (36.5%): alone in 17 and alongside other methods in 18. These two methodologies therefore accounted for antimicrobial interaction assessments in 72 (75%) of the publications.

Many investigators appear to be unaware of more sophisticated approaches to measurement of synergy and antagonism between antimicrobial compounds, although studies with antiviral agents almost all make use of the models developed by Chou & Talalay[1] or Prichard *et al.*,[2] both of which overcome many of the assumptions and limitations implicit in the chequerboard approach. The review by Greco *et al.*[3] is a very comprehensive account of most of the theoretical approaches to modelling drug interactive effects. In the course of this review, a single chequerboard dataset is analysed by many different mathematical models of interaction, leading to interpretations of synergy, antagonism and no interaction according to the model chosen.

It should not be news that experimentation on drug interactions can lead to opposite conclusions by different methodologies. The point has been made previously in reference books and in papers, such as the recent one by Lewis *et al.*[4] in the antifungal field. Of all methods available to test interactions, the FICI approach, so popular among bacteriologists and mycologists, is also possibly particularly prone to reproducibility problems; Rand *et al.*[5] found 25% of their replicate test sets gave discordant interpretations by FICI. Since there is a widely accepted norm in MIC testing, that variation in a single result places an MIC in a three-dilution range (mode ± 1 dilution), the possibilities for reproducibility errors in an MIC chequerboard are considerable.

For these reasons it is not rational for authors to make fine-scale interpretations of data from FICI experiments. Conclusions that interactions are 'additive', 'indifferent' or show 'partial synergy' applied to FICI data slightly above or below the critical theoretical cut-off of 1.0 seem to put a positive spin on findings that, within the limits of experimental error, really indicate only 'no interaction' between agents. In future, the *Journal of Antimicrobial Chemotherapy* will therefore insist that authors submitting papers containing FICI data restrict themselves to interpretations of 'synergy' (FICI ≤ 0.5), 'antagonism' (FICI > 4.0) and 'no interaction' (FICI > 0.5–4.0). This will encourage conservative interpretation of results and means that this journal's instructions on FICI interpretation are the same as those of other specialist journals in the antimicrobial field.

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