Technical methods

A simple test to demonstrate antibiotic synergism

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To be bactericidal, therapy of enterococcal endocarditis most often requires the combination of penicillin (or ampicillin) and an aminoglycoside. Therapeutic failures, however, have been encountered which could be attributed to a lack of synergistic effects between these two antibiotics (Weinstein and Moellering, 1973).

In vitro tests of antibiotic combinations generally deal with a bacteriostatic synergism. Study of a bactericidal synergism requires methods which are too time-consuming to be performed as a 'routine' service.

Using an agar triple layer technique (Schoutens and Yourassowsky, 1974) and enzymatic inactivation of penicillin-antibiotic (Yourassowsky and Schoutens 1974), as previously described, a simple method has been developed to demonstrate antibiotic synergistic effects which sometimes would not be detected by the classic disc method.

The media are poured into 10 cm diameter Petri dishes (fig 1). The bottom layer (layer 1) is constituted of 10 ml of Diagnostic Sensitivity Test Oxoid (DST) inoculated with 10^6 organisms. After cooling, this layer is covered with 10 ml of the same medium (layer 2); before complete solidification of layer 2, antibiotic discs (BBL) are pressed to the surface of layer 1. Discs are separated from each other by a distance which is equal to one-half of the sum of the inhibition zone diameters given by Bauer *et al* (1966) as cut-off points for antibiotic resistance.

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Fig 1 Triple layer technique: (1) inoculated layer; (2) median layer with antibiotic discs; (3) top layer containing penicillinase.

(Choice of these criteria is temporary. Investigations are in progress to define with more accuracy criteria which could allow quantitative evaluation of the synergism phenomenon.)

The plates are incubated at 37°C for 5 h, then covered with layer 3, which consists of 10 ml of DST agar to which, just before pouring, 0.75 ml of penicillinase is added (Bacto-Penase concentrate: 1 ml has a potency of at least 20 000 Levy units). Plates are placed back in the incubator for overnight incubation.

As an example, fig 2 shows the results of the study of synergism between ampicillin and gentamicin against a strain of *Streptococcus faecalis*. This shows (a) the weak bactericidal activity of ampicillin when acting alone for 5 h; (b) the lack of bacteriostatic synergism demonstrated by the stan-

Fig 2 Ampicillin-gentamicin synergism against *Str. faecalis*; (a) weak bactericidal activity of ampicillin when acting alone for 5 h; (b) no synergism demonstrated by the standard disc procedure; (c) strong synergism inside the zone where the two antibiotics are simultaneously present.

dard disc procedure; (c) the strong synergistic effects inside the zone where the two antibiotics are simultaneously present.

Results are easy to read, as are those obtained with penicillin G or other aminoglycosides (amikacin, gentamicin, streptomycin). Although less easy to read, similar results have been obtained by using the Kirby Bauer disc diffusion method (instead of the triple layer technique), penase solution being then directly set on the ampicillin disc after 5 h incubation.

A technique based on this principle has been recently published by Lee and Komarmy (1975).

It is highly probable that microorganisms situated inside the zone of synergism are killed; definite confirmation of this, however, would be brought about only by simultaneous inactivation of the two

antibiotics; an aminoglycoside-destroying enzyme, unfortunately, is not yet commercially available.

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A modified rack for the LKB Sample Processor

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The LKB Sample Processor (2071) is a programmable unit that can be adapted to many different tests in the laboratory. Racks carrying samples in disposable tubes are fed through a sampler unit. From here serum may be aspirated (in duplicate if desired) and dispensed with reagent or diluent into a second set of tubes in racks fed in parallel through the main unit. Both sets are moved by cogs that engage holes in the side of the racks. The tubes, projecting above the upper surface of the rack, activate a micro-switch, and operation ceases if no tube is present. Further reagents may be added by dispensing pumps to which reagent containers are attached.

The versatile nature of the system and the speed of operation (sampling speed of 400 per hour) recommends it as a radioimmunoassay sample processor and we have used it as such for over a year. There are two main problems. Many reagents for radioimmunoassay are bought in kit form: in the case of antisera and labelled antigen the volumes

supplied are small, often 5 or 10 ml, and just sufficient for the number of tests indicated. If these expensive reagents are dispensed through the pump, the deadspace of pump, tubing, and container may amount to 20% or more of the reagent with undesirable waste. A second problem is that one is reluctant to pass radioactive and proteinaceous material through a system with possible resultant contamination. The latter can, however, be overcome by thorough washing through after use.

Both of these disadvantages would disappear if one could repetitively aspirate the reagent from a container in the sampling position and dispense, with buffer, into the reaction tubes. The sample processor cannot be programmed to do this as the sampling mechanism will only operate if a sample rack, containing tubes, is in position and moves in parallel with the rack of recipient tubes.

We have overcome this problem by using a Teflon rack identical in length and width with the sample rack but with the following differences (figure):

1. It is as high as a rack loaded with tubes. This activates the tube-detecting microswitch.
2. Three cavities, of 5, 10, and 20 ml capacity, to contain reagents take the place of the holes for tubes.
3. At the level of the holes normally engaged by the cogs there is a full-length groove; thus the cogs of the sample operate but do not engage and move the rack.

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