Edited by
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Volume 2

Laboratory Aspects of Infections

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Preface

The International Society of Chemotherapy meets every two years to review progress in chemotherapy of infections and of malignant disease. Each meeting gets larger to encompass the extension of chemotherapy into new areas. In some instances, expansion has been rapid, for example in cephalosporins, penicillins and combination chemotherapy of cancer - in others slow, as in the field of parasitology. New problems of resistance and untoward effects arise; reduction of host toxicity without loss of antitumour activity by new substances occupies wide attention. The improved results with cancer chemotherapy, especially in leukaemias, are leading to a greater prevalence of severe infection in patients so treated, pharmacokinetics of drugs in normal and diseased subjects is receiving increasing attention along with related problems of bioavailability and interactions between drugs. Meanwhile the attack on some of the major bacterial infections, such as gonorrhoea and tuberculosis, which were among the first infections to feel the impact of chemotherapy, still continue to be major world problems and are now under attack with new agents and new methods.

From this wide field and the 1,000 papers read at the Congress we have produced Proceedings which reflect the variety and vigour of research in this important field of medicine. It was not possible to include all of the papers presented at the Congress but we have attempted to include most aspects of current progress in chemotherapy.

We thank the authors of these communications for their cooperation in enabling the Proceedings to be available at the earliest possible date. The method of preparation does not allow for uniformity of typefaces and presentation of the material and we hope that the blemishes of language and typographical errors do not detract from the understanding of the reader and the importance of the Proceedings.

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INTRODUCTORY COMMENTS

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SUMMARY

Improvements in susceptibility testing require better definition of reagents, test conditions, control strain maintenance and qualitative interpretative criteria. Quality control and external proficiency testing are keys to better performance at the operating level and will be discussed in the symposium. Developments in mechanization and automation are accelerating, and procedures designed to give results on the same day the test is set up are being developed. The need for agreed reference standards is again pointed up by these developments.

There have been increasing efforts in different countries during the past five or six years to improve regional performance of clinical susceptibility testing. Approaches have varied from attempts to improve reproducibility in absolute terms with methodological and reagent standardization to those which focus more on reproducibility of "qualitative" or "clinical" categorizations and on comparisons with control strains, with less emphasis on the procedure employed. Examples of both will be described in this symposium. Unfortunately, despite these advances, we are still in a situation in which agreement on reference dilution procedures has not been achieved and in which MIC results may differ considerably between procedures in different laboratories.

The fundamental problems with susceptibility testing have been the method dependency of results, the absence of fixed standards, and the lack of agreed criteria for interpretation. The results of both dilution and diffusion procedures are influenced, in varying degrees, by variations of inoculum, medium constitution, pH, and incubation

temperature and duration. In addition, diffusion test results are influenced by disc content and by any substantial growth rate differences in the organisms tested. Without agreed standards or procedures, it is therefore hardly surprising that interlaboratory reproducibility studies in the past showed some quite striking diversities (Hoffman, et al., 1958; Report, 1965; Survey, 1968; McCracken and Palmer, 1969; Ericsson and Sherris, 1971). That results were tolerable at all was probably due to the fact that many pathogens are often highly susceptible or highly resistant to the earlier introduced antimicrobics, and these properties frequently permitted discrimination with even poorly standardized test systems. Clear cut bimodal distributions of susceptibility are less marked or absent with other antimicrobics such as the cephalosporins and broader spectrum penicillins, and with some species which are now seen more commonly in infections in the compromised host. These considerations increase the importance of higher levels of reproducibility.

Better absolute reproducibility can be obtained. Test conditions can be standardized including such components as medium volume, pH, incubation conditions, etc. Antimicrobic disc contents, which were shown to vary excessively in the past in North America (Branch et al., 1957; Greenberg et al., 1957) can be controlled. They are now subject to batch certification regulations in North America and their performance can be assured within established limits (which could still be tightened). Endpoint reading is more prone to subjectivity, but difficulties can be reduced by establishing prefixed criteria, sometimes by simple manipulations such as reading diffusion plates through the back rather than the front, or by reading broth endpoints with relatively simple photometric devices. Inocula can be standardized with various levels of precision depending on the care which is expended or the equipment that is available. In recent studies in our laboratories by Dr. Marie Coyle, duplicate tests with two readers on two successive days were made of a large number of isolates by means of an agar overlay technique using a photometrically adjusted inoculum. Among 294 sets of 8 observations, only 8 (2.7%) had a range of >3 mm for the 8 readings, a level of precision which is considerably greater than we have achieved before.

The largest single problem in achieving reproducibility in absolute terms remains the medium. Individual products of single manufacturers usually show good reproducibility from batch to batch (Brenner and Sherris, 1972; Barry and Effinger, 1974); however, as will be considered elsewhere in this meeting, products of some manufacturers may yield results considerably different from those of others, particularly with antimicrobics affected by divalent cations or by total salt content. These include the aminoglycosides, the tetracyclines, and the polymyxins. Similarly, differences in thymidine content of media may substantially influence the endpoint clarity and absolute results with the sulfonamides and trimethoprim (R. Ferone et al., 1975).

Intermanufacturer reproducibility of presently used media could be greatly improved if performance limits were established. This should not be too burdensome because batches can be adjusted to meet particular performance requirements. For example, divalent cation content can be manipulated to adjust the gentamicin vs. Pseudomonas aeruginosa performance of MH media of low magnesium and calcium content to conform to a reference batch or yield results within a predetermined range with control strains (Reller et al., 1974; Sherris and Aitken, 1975, unpublished data). Improvement in performance of complex media with sulfonamides and trimethoprim can be attained by enzymic inactivation of thymidine (R. Ferone et al., 1975). Clearly, however, the problem would be greatly simplified with the use of better defined media at least to the point of full descriptions of manufacturing procedures of complex components such as peptones.

In studies by an international collaborative group, it was shown that even when the same medium, reagents, and strains are tested with a standard protocol, there can be very substantial variation between the results of different laboratories (Ericsson and Sherris, 1971). This illustrated clearly the need for standard or control strains, and Chabbert defined the criteria for their selection (Chabbert, 1971). Specific recommendations for control strains have now been made in different countries, but their value depends on their stability under storage conditions. Coyle has recently compared 15 strains of S. aureus and E. coli, which were sent to her by laboratories in different parts of the United States. Each organism was originally derived from the "Seattle" strain (ATCC 25923 and 25922). The strains were maintained under a variety of conditions, some of which were suboptimal. All were tested in duplicate on two occasions by the diffusion test mentioned above, and compared with a strain derived directly from ATCC. Among the staphylococci, one was a penicillinase producer of different phage type to the ATCC strain. All others were of the same phage type and gave zone diameters within 2 mm of the standard with all antimicrobics except the penicillins and cephalothin. Three strains gave penicillin zones which exceeded the others by 3.5 - 7 mm, although all were within the FDA performance range (Fed. Reg., 1972; Fed. Reg., 1973). One strain of E. coli differed in biotype from the ATCC strain and one gave several divergent results. The remaining 13 yielded only 4 results among 113 which differed from the ATCC strain by more than 1 mm, and none that differed by 2 mm or more. Reller (1974) made deliberate attempts to cause variation in proposed Pseudomonas aeruginosa control strains by repeated subculture under adverse conditions and failed. It thus appears that quite high levels of reproducibility of performance with standard strains can be obtained under operating conditions in different laboratories if storage and use conditions are well defined, and this is obviously critical for methods that depend on results relative to standard strains as well as for quality control of procedures that seek reproducibility in absolute terms.

The ultimate test of success in improving susceptibility test performance is at the operating level in the clinical or public health laboratory and several papers in this symposium bear on quality control and performance evaluation. With procedures in which reproducibility is sought in absolute terms, performance limits for control strains need to be established and met within the laboratory. For all procedures, external proficiency test evaluation is an invaluable adjunct and is now being used increasingly. Not only can it measure acceptable performance in relationship to results of reference or referee laboratories, but a detailed analysis of the results can often indicate the nature of the problem in an individual laboratory with poor performance. Performance evaluation systems in clinical microbiology generally can be a most effective educational tool when there is immediate follow-up explanations of sources of error.

Interpretative categories for susceptibility test results have to be determined from several considerations. Relationship of in vitro inhibitory concentrations to blood or other body fluid levels are useful, but when taken alone can be a considerable oversimplification. Distribution of susceptibilities among strains or variants of known responsiveness must also be considered. The borderlines of categories are ultimately best judgment decisions, taking into account these several components. They should be agreed and clearly defined by workers with special knowledge in the field. There have been few well designed studies to test the validity of particular categorical schemes, although much clinical operating experience has been gained. An essential prerequisite to better controlled studies on new agents is methodological reproducibility to provide a better data base.

While work has been continuing in standardization and performance evaluation of traditional methods, there have been a number of new developments towards automated and mechanized procedures (Automation, 1975) and commercially produced test systems which only require inoculation. Some of these have considerable promise and some would appear to be modifiable so that their benefits could be achieved without the use of expensive hardware. Such procedures should reduce the technical errors associated with "hand" methods. Devices now available or under development include procedures for automatically distributing pre-fixed broth volumes, adding antimicrobics, usually as elution discs, and for reading results photometrically or by changes in electrical impedence (Automation, 1975). Automatic particle counting devices have also been developed for measuring microbial growth or inhibition (Isenberg et al., 1971). It seems probable that many devices can be developed to the point of significant utility and accuracy for susceptibility testing in relationship to established methods if overnight reading is employed. Their greatest potential use, however, is in providing results on the day on which the test is set up. To date, efforts to equate rapid to overnight results have focussed on modifying the concentrations of antimicrobic used in the test. Results in our laboratory indicate

that better equivalence will result by using heavier inocula for the early-read test, and we believe this approach merits further exploration (Lampe et al., 1975). The need for reference standards is again clear.

Despite these developments, diffusion testing is likely to remain a mainstay for smaller laboratories for several years to come. Performance can be improved and more selective use could almost certainly offset any added cost of better reagents, quality control and proficiency testing. It is to be hoped that the interchange of information and experience in this symposium will be an added stimulus to the further work that is needed to continue to improve susceptibility testing methodology and utility.

REFERENCES

Automation in microbiology and immunology (1975), Hedén, C.-G., and T. Illéni, eds. John Wiley & Sons, Publishers.

Barry, A. L., and Effinger, L. J. (1974), Am. J. Clin. Pathol., 62, 113.

Branch, A., Starkey, D. H., Power, E. E., and Greenberg, L. (1957), In Antibiotics Annual 1956-57. Antibiotica, Inc., N.Y., p. 898.

Brenner, V. C., and Sherris, J. C. (1972), Antimicrob. Ag. Chemother., 1, 116.

Chabbert, Y. A. (1971), In Ericsson, H. M., and Sherris, J. C. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. et Microbiol. Scandinav., Section B, Suppl. No. 217.

Ericsson, H. M., and Sherris, J. C. (1971), Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. et Microbiol. Scandinav., Section B, Suppl. No. 217.

Federal Register (1972), Rules and regulations. Antibiotic susceptibility discs. Fed. Regist. 37, 20525-20529.

Federal Register (1973), Rules and regulations. Antibiotic susceptibility discs--correction. Fed. Regist. 38, 2576.

Ferone, R., Bushby, S. R. M., Burchall, J. J., Moore, W. D., and Smith, D. (1975), Antimicrob. Ag. Chemother., 7, 91-98.

Greenberg, L., Fitzpatrick, K. M., and Branch, A. (1957), Canad. M. A. J., 76, 194.

Hoffman, R. V., Jr., Jackson, G. G., and Turner, M. P. (1958), J. Lab. Clin. Med., 51, 873.

Isenberg, H. D., Reichler, A., and Wiseman, D. (1971), Appl. Microbiol., 22, 980.

Lampe, M. F., Aitken, C. L., Dennis, P. G., Forsythe, P. S., Patrick, K. E., Schoenknecht, F. D., and Sherris, J. C. (1975), Antimicrobial Ag. Chemother., in press.

McCracken, L. M., and Palmer, P. H. (1969), N. Z. med. J., 70, 390.

Reller, L. B., Schoenknecht, F. D., Kenny, M. A., and Sherris, J. C. (1974), J. Infect. Dis., 130, 454-463.

Report on antibiotic sensitivity test trial organized by the Bacteriology Committee of the Assoc. of Clin. Pathologists (1965), J. clin. Path., 18, 1.

Survey of antibiotic sensitivity testing (1968), Med. J. Aust., 2, 171.