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In the words of the authors, the paper by A. W. Bauer et al., from the University of Washington in Seattle, on a standardized single-disk method for antibiotic susceptibility testing ". . . consolidate(s) and update(s) previous descriptions of the method and provide(s) a concise outline for its performance and interpretation." Clinical microbiologists were relieved that finally a disk diffusion method had been standardized, could be used with ease, and provided reliable results as compared with minimum inhibitory concentration tests. The pivotal role of Hans Ericsson's theoretical and practical studies (H. Ericsson and G. Svartz-Malmberg, *Antibiot. Chemother.* **6:**41–74, 1959), as well as earlier reports by some of the authors of the publications cited, must be mentioned as a matter of fairness. Most of the recommendations given are still valid today even though some of the antimicrobial agents are obsolete, new ones have been added, some zone sizes had to be modified, and new media were designed for *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Recommendations of the National Committee for Clinical Laboratory Standards continue to be based on this publication; the "Kirby-Bauer" method is, among the many disk methods used in other countries, still the one that has been researched most thoroughly and updated continuously.

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ANTIBIOTIC SUSCEPTIBILITY TESTING BY A STANDARDIZED SINGLE DISK METHOD

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Most clinical microbiologic laboratories in this country now use the paper disk method for determining susceptibility of bacteria to antibiotics and chemotherapeutic agents. A number of modifications of the test are employed. When this type of test was first developed, only 1 disk was used for each agent to be tested,[7, 10] but subsequently it became common practice to use 2 or more disks of different potency and to judge susceptibility on the basis of the presence or absence of growth around the disks. Our approach has been to continue to develop a single disk method based on measurement of sizes of zones. We believe that this is rational in theory and that it correlates better with the results of dilution technics.

A number of reports on the technical details, experimental basis, and interpretative standards of the single disk method have been published,[1, 4-6, 13] and recently some of the theoretical aspects have been reviewed in more detail.[2, 3, 11] The purpose of the present communication is to consolidate and update previous descriptions of the method and provide a concise outline for its performance and interpretation.

METHOD

Rapidly Growing Pathogens Such as Staphylococci and Enterobacteriaceae

A few colonies (3 to 10) of the organism to be tested are picked with a wire loop from the original culture plate and introduced into a test tube containing 4 ml. of tryptose phosphate or trypticase soy broth. These tubes are then incubated for 2 to 5 hr., to produce a bacterial suspension of moderate cloudiness. The suspension is then diluted, if necessary, with water or saline solution to a density visually equivalent to that of a standard prepared by adding 0.5 ml. of 1 per cent $BaCl_2$ to 99.5 ml. of 1 per cent H_2SO_4 (0.36 N). An alternative procedure is to dilute broth cultures overnight to the density of the opacity standard (10- to 100-fold). For the sensitivity plates, large (15-cm.) Petri dishes are used with Mueller-Hinton agar (5 to 6 mm. in depth). Plates are dried for about 30 min. before inoculation and are used within 4 days of preparation.

The bacterial broth suspension is streaked evenly in 3 planes onto the surface of the medium with a cotton swab (not a wire loop or glass rod). Surplus suspension is removed from the swab by being rotated against the side of the tube before the plates are seeded. After the inoculum has dried (3 to 5 min.), the disks are placed on the agar with flamed forceps or a single disk applicator and gently pressed down to ensure contact. Plates are incubated immediately, or within 30 min. The large Petri dishes are spacious enough to accommodate about 9 disks in an outer ring, and 3 or 4 more in the center. It is advantageous to place antibiotics which diffuse well in the outer circle and disks which produce smaller inhibition zones, such as vancomycin and polymyxin-B, in the central area of the plate.

After overnight incubation, the zone diameters (including the 6-mm. disk) are measured with a ruler on the undersurface of the Petri dish or with calipers near the agar surface. A reading of 6 mm. indicates no zone. The end point is taken as complete inhibition of growth as determined by the naked eye, except in the case of sulfonamides, where organisms grow through several generations before inhibition takes effect.

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TABLE 1

ZONE SIZES AND THEIR INTERPRETATION FOR FREQUENTLY USED CHEMOTHERAPEUTICS

Antibiotic or Chemotherapeutic Agent	Disk Potency	Inhibition Zone Diameter to Nearest Millimeter		
		Resistant	Intermediate	Sensitive
Ampicillin				
S. aureus	10 μg.	20 or less	21–28	29 or more
All other organisms	10 μg.	11 or less	12–13	14 or more
Bacitracin	10 units	8 or less	9–12	13 or more
Cephalothin	30 μg.	14 or less	15–17	18 or more
Chloramphenicol	30 μg.	12 or less	13–17	18 or more
Colistin	10 μg.	8 or less	9–10	11 or more
Erythromycin	15 μg.	13 or less	14–17	18 or more
Kanamycin	30 μg.	13 or less	14–17	18 or more
Lincomycin§	2 μg.			17 or more
Methicillin	5 μg.	9 or less	10–13	14 or more
Nalidixic acid*	30 μg.	13 or less	14–18	19 or more
Neomycin	30 μg.	12 or less	13–16	17 or more
Nitrofurantoin*	300 μg.	14 or less	15–16	17 or more
Novobiocin†	30 μg.	17 or less	18–21	22 or more
Oleandomycin	15 μg.	11 or less	12–16	17 or more
Penicillin-G	10 units	20 or less	21–28	29 or more
Polymyxin-B	300 units	8 or less	9–11	12 or more
Streptomycin	10 μg.	11 or less	12–14	15 or more
Sulfonamides‡	300 μg.	12 or less	13–16	17 or more
Tetracycline	30 μg.	14 or less	15–18	19 or more
Vancomycin	30 μg.	9 or less	10–11	12 or more

* Standards apply to urinary tract infections only.

† Zone sizes not applicable when blood is added to medium.

‡ Any of the commercially available 300- or 250-μg. sulfonamide disks may be used with the same standards of zone interpretation.

§ Tentative standard.

Slight growth (80 per cent or more inhibition) with sulfonamides is therefore disregarded, and the margin of heavy growth read to determine the zone size.[6] Swarming of Proteus strains is not inhibited by all antibiotics, and a veil of swarming into an inhibition zone is also ignored. If several colonies are seen within a zone of inhibition, the strain should be checked for purity and retested. If they are still present, the colonies are regarded as significant growth; this is not a common occurrence. The zone diameters are recorded and interpreted according to Table 1, and the results are reported to the clinician. When they are needed, zone diameters may be read after incubation for 6 to 8 hr. Standard control organisms of known susceptibility should be employed at least once a week as a check on the activity of the disks and on the reproducibility of the test.

The technic has proved satisfactory for sulfonamide susceptibility testing of all organisms examined except Group A β-hemolytic streptococci; these do not show clear cut-zones and the method should not be used for the organism.

Slower Growing and Fastidious Organisms

The standards in Table 1 have been developed for rapidly growing pathogens, for which the test is highly satisfactory. More slowly growing organisms, such as Hemophilus, have been less completely studied, but zone sizes are somewhat larger for an equivalent minimum inhibitory concentration than they are with rapid growers. For testing such organisms, 5 per cent sheep or human blood may be added to the medium, which may also be "chocolatized" when indicated.

Organisms of this type, with zone sizes in

the resistant range, may be safely reported as resistant. Those giving zones in the intermediate range or within 2 mm. of the borderline between sensitive and intermediate should be regarded as of uncertain susceptibility and tested by a dilution method if one is indicated.

The test cannot be regarded as more than a very rough guide for organisms requiring more than 24 hr. to yield macroscopic colonies. It should not be used for sensitivities on *Neisseria gonorrhoeae* or for sulfonamide sensitivities on meningococci until further data become available.

DISCUSSION

The numerical values in the table have been established by comparing zone sizes with a large series of tube or plate dilution tests and by relating these to blood levels found with frequently used dose schedules. In the case of nitrofurantoin and nalidixic acid, levels in the urinary tract have been taken into account in establishing standards. Confirmation of the validity of these standards has also been obtained from curves showing the distribution of susceptibilities of large numbers of individual strains of various species.[3] With many species from which resistant mutants have emerged, such distribution curves show 2 rather clearly separated sensitive and resistant populations, with few strains falling in the intermediate zone. For example, not more than 3 to 5 per cent of all strains of *Staphylococcus aureus* fall into the intermediate range with tetracycline, chloramphenicol, or erythromycin.

The technic should be used exactly as described, because although it has considerable flexibility, changes in conditions may combine to produce inaccuracies; it should be particularly stressed that the zone sizes in the table are *only applicable to the use of Mueller-Hinton medium* and to disk contents listed in the table. Undiluted overnight broth cultures should never be used as an inoculum, but diluted at least 10-fold, or preferably to a density equivalent to the barium sulfate standard. Growth by this method should be confluent with a properly standardized inoculum, and the test must be repeated if this is not achieved.

The choice of antibiotics to be tested depends on a number of factors, such as the type of practice of the laboratory and the local preference for a particular agent. For most Gram-positive organisms, the disks routinely used in our laboratories are penicillin-G, methicillin, tetracycline, erythromycin, streptomycin, chloramphenicol, kanamycin, and, occasionally, sulfamethizole and ampicillin. Gram-negative rods are tested with chloramphenicol, tetracycline, streptomycin, kanamycin, polymyxin-B, sulfamethizole, ampicillin, and cephalothin, with added nitrofurantoin for urinary tract infections. Only 1 penicillinase-resistant penicillin and 1 of the tetracyclines are tested routinely because of essential cross-resistance within these groups. Some organisms may be tested only against antibiotics to which resistance has developed; thus Group A β-hemolytic streptococci and pneumococci may need to be tested only against tetracycline. Disks from first line supply houses which are tested and certified for potency by the Food and Drug Administration have proven reliable. They should be stored exactly as recommended.

It will be noted that the interpretative zone diameters are different for each agent, not only because the disk potencies vary, but also because the diffusion and solubility properties of the drugs in Mueller-Hinton medium are different and characteristic for each agent. Obviously, the disk producing the largest inhibition zone does not necessarily indicate the antibiotic of choice for a given pathogen. If, for instance, a strain of *Escherichia coli* shows a zone of 22 mm. around a 30-μg. tetracycline disk and a zone of 26 mm. around a 30-μg. chloramphenicol disk, all that can be said is that the strain is susceptible to both compounds. From the agents reported as being effective against a given organism, the clinician may select the one most appropriate for therapy of the particular case. Pharmacologic and toxicologic properties, as well as the bacteriostatic or bactericidal actions of the drugs, will be taken into account in making this selection. A report of "intermediate" or "resistant"

does not necessarily imply that treatment may not be successful with unusually high dosage. Useful predictions of the efficacy of such treatment, however, can only be obtained from comparisons of the results of dilution technics with anticipated chemotherapeutic levels at the sites of infection.

It will also be seen that the sensitivity criteria given for *S. aureus* with ampicillin are different from those of other organisms, because some weakly penicillinase-producing strains of staphylococci may give zone sizes of more than 14 mm., although they should be regarded as clinically resistant. All non-penicillinase-producing staphylococci have zones similar to those seen with penicillin.

The importance of standardizing test conditions as far as possible in practice cannot be overstressed. For this reason, we do not recommend routine direct application of disks to plates seeded with clinical material because of problems of inoculum control and mixed cultures. In emergency situations, such as those in which direct smears of some cerebrospinal fluid and urine specimens indicate that a pure culture may be anticipated, we make direct tests and issue a tentative report, which is always then confirmed with the standard method. Similar emphasis on standardization has been made by Ericsson[8, 9] in his description of an excellent single disk technic which is widely used in Scandinavia, and has been stressed in the Second Report of the Expert Committee on Antibiotics of the World Health Organization,[12] which is sponsoring further studies towards the possible establishment of internationally acceptable standards. Such standards are highly desirable and may lead us to some modifications of our method. In the interim, however, we have found the technic to be easily performed, reproducible, and of great value as a guide to therapy.

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