A SIMPLE METHOD FOR THE DETERMINATION OF ANTIBIOTIC SENSITIVITY

AN ADAPTATION OF THE FILTER PAPER METHOD

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The method to be described is based on the observation that the diffusion of the antibiotic from impregnated filter paper into the underlying medium is achieved in a very short time. Experiments were performed to show the influence on the size of the inhibition zone of the period of contact of the antibiotic strip with the medium. The results are shown in Fig. 1. Identical results were obtained with other antibiotics and with various organisms.

It is therefore immaterial whether the filter paper remains in contact with the medium for a period longer than a certain minimum. It also does not affect the result whether the strip is left for the whole incubation period or removed before inoculation (Fig. 2).

These results are in agreement with those obtained by Jensen and Kioer (1948) and Mørch-Lund (1949) who used wet antibiotic discs. Kokko (1947) and Evans (1948) made similar observations with regard to sulphonamides. These authors, however, regarded five hours as a suitable time of contact.

Materials and Technique

Filter Paper.—Ford's coloured filter papers have been used throughout. The filter paper is cut into shapes which fit into the plate and are suitable for transverse streaking of the test organisms. Segments of a circle and oblong strips serve this purpose best.

The filter papers are impregnated in the usual manner. Both wet and dry strips have been used with equal success, with the exception of dry aureomycin strips, which were not satisfactory. The concentrations of test solutions used as routine were: for wet strips, penicillin, 20 u./ml.; streptomycin, 250 u./ml.; chloromycetin, 50–250 µg./ml.; aureomycin, 50–250 µg./ml. For dry strips the concentra-

tions were: penicillin, 50–100 u./ml.; streptomycin, 500 u./ml.; chloromycetin, 500 µg./ml.

The concentration of antibiotic solutions used for impregnation of the filter paper strips should be kept as low as is compatible with clear results. Thus one of the possible sources of inaccuracy of the diffusion methods—namely, that resulting from a steep diffusion gradient—can be minimized and a closer estimate of the inhibiting concentration of the antibiotic can be obtained.

Standard Organisms.—As a rule an Oxford *H. staphylococcus* was used. A sensitive strain of *Bact. coli* was used for streptomycin and chloromycetin sensitivity tests on McConkey's medium, particularly in cases of urinary infection.

Preparation of the Plate.—A filter paper strip impregnated with the desired concentration of the antibiotic test solution is placed on the surface of the medium, left there for any convenient period between 30 minutes and 12 hours, and removed before inoculation. This applies both to wet and dry strips. For wet strips even 10 minutes is quite sufficient to obtain adequate diffusion.

Usually the plates are placed in the incubator, as this also dries them. If wet strips are used it is advisable to dry the plate for a short time before inoculation, so as to avoid any carrying over of the antibiotic solution which may have been left on the surface. For routine purposes it has been found quite convenient to prepare a number of sensitivity plates with the most commonly used antibiotics in the morning, remove the strips after about 30 minutes, and have the plates ready for use during the course of the day. If some plates are left over they can be used the next day.

Inoculation.—The "multiple streak method" was employed as a rule. The organism to be tested is streaked across the plate at right angles to the antibiotic zones. Using this method, linear inhibition

FIG. 1

FIG. 2

FIG. 3

zones are obtained which are readily compared and easy to interpret.

A standard organism of known sensitivity is invariably streaked on each plate or half plate if only half a plate is used for one determination. The presence of a standard organism inoculated under the same conditions is absolutely essential. It provides a standard and measure of comparison and at the same time acts as a control of the efficiency of the antibiotic strip employed. The size of the inoculum is,

FIG. 1.—Wet strip impregnated with 10 u./ml. penicillin. Portions removed after (1) 10 minutes, (2) 1 hour, (3) 4 hours, (4) 12 hours. Medium was sliced to prevent cross diffusion. After removal of the last portion all areas were streaked simultaneously with *Staph. pyogenes*.

FIG. 2.—Wet antibiotic strip impregnated with 10 u./ml. penicillin cut in two halves. The top half plate was inoculated after one of the half strips was applied for 30 minutes and removed. The bottom half plate was inoculated first and the second half strip applied and left for 24 hours. Ox=Oxford staphylococcus. R. St.=resistant *Staph. pyogenes*. S. St.=sensitive *Staph. pyogenes*.

FIG. 3.—Antibiotic spectrum on primary culture. Material was a swab from an ulcer of the leg. Dry strips were applied and removed before inoculation. Organisms were penicillin-resistant *Staph. pyogenes* and diphtheroids, partly resistant to streptomycin (small grey colonies) (aureomycin wet strip). Plate bisected by gutter.

P=Penicillin. S=Streptomycin. C=Chloromycetin. A=Aureomycin.

The shaded area is the surface of the medium in contact with the strip before inoculation.

of course, one of the most difficult factors to control. In order to obtain the most satisfactory result it is advisable to start with a heavy inoculum and thin it out by parallel streaking so that the inhibitory effect of the same concentration of the antibiotic on different size inoculum can be observed at the same time. Plates are incubated for about 16 to 24 hours.

Primary Culture and Sensitivity Determination.

The method described lends itself very well to primary culture combined with a determination of antibiotic

sensitivity. The sensitivity plate is prepared in such a way that only part of it is affected by the antibiotic, the rest of the plate surface forms an ordinary culture plate (Fig. 3).

The material to be cultured is first rubbed across the plate at right angles to the antibiotic zones and then it is thinned out, as in an ordinary culture. The heavier inoculated streak shows the sensitivity even if only scanty organisms are present. A standard organism is always included.

In urinary infections, primary culture and sensitivity tests on a McConkey plate give satisfactory and reliable results. This procedure saves an appreciable amount of time.

Results and Discussion

The method can be used for testing sensitivity to any antibiotic diffusible in agar. It combines certain advantages of the ditch plate method with the ease and simplicity of the filter paper method. The inhibition zones are usually clear cut and easily compared at a glance with the standard organism, the sensitivity of which is known. The linearity of the inhibition zones makes this comparison more obvious. As the standard organism is inoculated on the same plate and under exactly the same condition, a comparison provides a reliable guide. There is no creep along the edges of the filter paper, as happens with some organisms, e.g., *Ps. pyocyanea*.

An economy of media is achieved as eight tests can be easily performed on the same plate (four organisms against two antibiotics). If the plate is cut in half, even four antibiotics can be tested, thus giving a "spectrum" for most of the antibiotics commonly employed (Fig. 3).

It is well understood that the method shares the relative inaccuracy of other diffusion methods, and the filter paper method in particular.

Summary

A method for the determination of antibiotic sensitivity is described and its application in routine examinations discussed. The procedure is an adaptation of the filter paper method and consists in applying impregnated filter paper strips to the surface of the medium for a short time and removing it before inoculation. By cutting the filter paper to a suitable shape and streaking the material across the plate, linear inhibition zones are obtained. The sensitivity is estimated by comparison with a standard control organism of known sensitivity. The method proved its value in routine work. It is sufficiently accurate, simple, economical, and time saving.

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Addendum

Since this paper was accepted for publication an alternative technique has been tried and found very satisfactory. It consists in the application of the antibiotic filter paper strips *after* the medium has been inoculated. The strips are then removed after a period of 30 minutes to one hour. In all other respects the technique is similar to that described in this paper.

Provided that the filter papers are carefully lifted from the agar, there is no danger of cross-contamination in this short period. One advantage of this technique is that it is not necessary to prepare the sensitivity plates in advance.