

DRIED DISC TECHNIQUE FOR DETERMINING SENSITIVITY TO THE ANTIBIOTICS

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Since the introduction of the antibiotics, the disc technique has been used by various workers for their assay (Vincent and Vincent, 1944 ; Epstein, Foley, Perrine, and Lee, 1944 ; Heatley, 1944), and also for the detection of bacterial sensitivities (Morley, 1945).

An attempt has been made to standardize the method of preparing and storing the disc impregnated with antibiotic to determine bacterial sensitivity within 18 hours' incubation. To accomplish this the discs must give a constant standard zone of growth inhibition when tested with a known sensitive organism, be conveniently prepared and dried, and have a sufficiently high concentration of the antibiotic impregnated into them to remain stable when stored.

Each disc is a standard size and is impregnated with a standard volume and concentration of the antibiotic. It was found, after experimenting with various-sized discs, that a 9 mm. disc cut from "No. 633 Hayle Mills" blotting-paper would absorb approximately the whole volume of one drop (0.02 ml.) from a 50-dropping pipette. As the discs are dried after impregnation by leaving them in an open incubator for two hours, it was found necessary to use a much higher concentration of antibiotic than has been used by previous workers (Morley, 1945). The concentration ultimately used gave a zone sufficiently large for measurement, and, at the same time, without a tendency to inhibit completely the growth of the pool inoculum.

Neither the size of the inoculum nor the degree of moisture on the plates seems to have an appreciable effect on the zoning. It has been found necessary to place the discs in an optimum position (Fig. 1), first, to avoid completely inhibiting the growth of

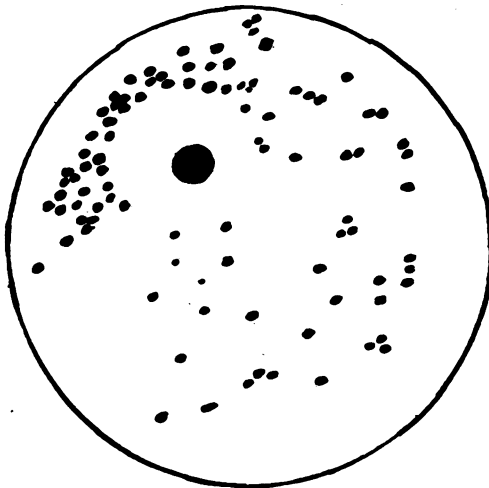


FIG. 1.—Diagram showing disc in optimum position to prevent complete inhibition of growth when inoculum is small.

inoculum and, secondly, to show the sensitivity of isolated colonies. The disc should not be less than 15 mm. from the edge of the plate, as there appears to be a reflection wave effect from the edge of the plate which gives inaccurate zoning. The discs cannot be used on penicillinase plates; in cases treated with penicillin a primary isolation of bacteria has to be made before discs can be used for testing their sensitivity.

Dried Penicillin Discs

Method of Preparation.—Using “No. 633 Hayle Mills” blotting-paper, discs are cut out with a 9 mm. cork borer, and are then separated out in a large size petri dish and sterilized in a hot air oven. Each disc is then impregnated with 1 drop (0.02 ml.) of a freshly made solution of 1,000 units penicillin per ml. using a 50-dropper pipette. The discs are dried in an incubator for two hours with the lid of the petri dish slightly open. Any moisture forming in the lid of the petri dish is removed with sterile filter paper and the discs are then stored in the refrigerator. They are stable for six to eight months.

Sensitivity Interpretation.—Measurements are made from the edge of the disc to the line of growth.

Examples.—*Staph. Mayo* gives 15 mm. = 0.025 unit per ml. tube sensitivity sensitive organism

Staph. 25W (Watford strain) gives 8 mm. = 0.25 unit per ml. tube sensitivity ∴ moderately sensitive organism.

Staph. 16X (Watford strain) gives 3 mm. = 0.3 unit per ml. tube sensitivity ∴ slightly sensitive organism.

Staph. 19BY (Watford strain) gives nil = 0.5 unit per ml. tube sensitivity ∴ resistant organism.

Dried Streptomycin Discs

Method of Preparation.—Discs are prepared and sterilized as for penicillin, and are then impregnated with 10,000 μg . per ml. streptomycin and dried as before. The discs are stable for about six months.

Sensitivity Interpretation.—Measurements are made from the edge of the disc to the line of growth.

Examples.—*Bact. coli* (Watford strain) gives 12 mm. = 10–5 μg . per ml. tube sensitivity ∴ sensitive organism.

Micrococcus 14W (Watford strain) gives no zone = 200 μg . per ml. tube sensitivity ∴ resistant organism.

Dried Aureomycin Discs

Method of Preparation.—Discs are prepared and sterilized as for penicillin and are then impregnated with a 2,500 μg . per ml. solution of aureomycin, and dried as before. The 2,500 μg . per ml. solution of aureomycin was prepared by dissolving the hydrochloride salt in sterile distilled water, buffered with acetate at pH 5. Owing to the instability of aureomycin in solution, reliable methods for tube sensitivity testing were not found, but although the values given are not absolute, they are at least relative, and in point of fact do correspond respectively with the zoning. These discs to date have been stable for six months.

Sensitivity Interpretation.—Measurements are made from the edge of the disc to the line of growth.

Examples.—*Staph. Mayo* gives 12 mm. = 0.25 μ g. per ml. tube sensitivity \therefore sensitive organism.
Bact. coli (Watford strain) gives 3 mm. = 2.5 μ g. per ml. tube sensitivity \therefore moderately sensitive organism.
Proteus (Watford strain) gives nil = 20 μ g. per ml. tube sensitivity \therefore resistant organism.
Staph. 19BY (Watford strain) gives 12 mm. = 0.25 μ g. per ml. tube sensitivity \therefore a sensitive penicillinase-producing *Staph.*

Dried Chloromycetin Discs

Method of Preparation.—Discs are prepared and sterilized as for penicillin and are then impregnated with a solution of 2,500 μ g. per ml. of chloromycetin and dried as before. To date the discs have been stable for two months.

Sensitivity Interpretation.—Measurements are made from the edge of the disc to the line of growth.

Examples.—*Staph. Mayo* gives 11 mm. = 3.1 μ g. per ml. tube sensitivity \therefore sensitive organism.
Bact. coli (Watford strain) gives 9 mm. = 6.2 μ g. per ml. tube sensitivity \therefore moderately sensitive organism.
Proteus (Watford strain) gives 8 mm. = 12.5 μ g. per ml. tube sensitivity \therefore slightly sensitive organism.

Table I analyses quantitatively certain bacterial sensitivities to penicillin, streptomycin, aureomycin, and chloromycetin.

TABLE I
AN APPROXIMATE QUANTITATIVE EVALUATION OF BACTERIAL SENSITIVITY TO ANTIBIOTICS

Anti-biotic	Concentration in Disc	Test Organism	Zoning (mm.)	Corresponding Tube Sensitivity	Sensitivity
Penicillin	1,000 units per ml.	<i>Staph. Mayo</i>	15	0.025 unit per ml.	Sensitive
		<i>Staph. 25W</i>	8	0.25 „ „	Moderately sensitive
		<i>Staph. 16X</i>	3	0.3 „ „	Slightly sensitive
		<i>Staph. 19BY</i>	0	0.5 „ „	Resistant
Streptomycin	10,000 μ g. per ml.	<i>Bact. coli</i>	12	10–5 μ g. per ml.	Sensitive
		<i>Micrococcus 14W</i>	0	200 „ „	Resistant
Aureomycin	2,500 μ g. per ml.	<i>Staph. Mayo</i>	12	0.25 „ „	Sensitive
		<i>Bact. coli</i>	3	2.5 „ „	Moderately sensitive
		<i>Proteus</i>	0	20 „ „	Resistant
		<i>Staph. 19BY</i> (penicillinase-producer)	12	0.25 „ „	Sensitive
Chloromycetin	2,500 μ g. per ml.	<i>Staph. Mayo</i>	11	3.1 „ „	„
		<i>Bact. coli</i>	9	6.2 „ „	Moderately sensitive
		<i>Proteus</i>	8	12.5 „ „	Slightly sensitive

Summary

A description is given of a method of determining the sensitivities of bacteria to the antibiotics by means of impregnated and dried blotting-paper discs. The method aims at standardizing the interpretation of this disc method and is for use in a routine laboratory, the discs being applied to the plates that have been sown direct with the material under investigation.

A zone of inhibition is given which is related to the concentration of the antibiotic necessary to inhibit growth. *Measurement of the zones is a fairly accurate guide to the sensitivity of the organism*, and therefore to the adequacy of the antibiotic dosage.

The discs are easily prepared and dried, convenient to handle, being immediately ready for use, and can be stored without loss of potency within a known period of time.

I should like to thank Drs. Stokes and Schwabacher, in whose laboratories this work was originally done. I should also like to thank Dr. Jones for his assistance.

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