In the Laboratory

A Method for Determining Bacterial Resistance and Susceptibility to Sulfonamides and Penicillin

ROGER D. REID, LT. COL., SN.C., O.R.C., and DOROTHY TROTTER ANDERSON

Eighth Service Command Laboratory, Fort Sam Houston, Texas

Wilson (*2*) has described a method for *in vitro* testing of the resistance of Group A hemolytic streptococci to sulfonamides. Although his technic has several advantages over earlier methods, the medium he describes is extremely complicated and the average laboratory does not have the materials at hand.

The necessity for routine determination of sulfonamide resistance or susceptibility of various pathogenic bacteria caused us to attempt a modification of Wilson's medium. The results we have obtained are entirely satisfactory, yet the test is extremely simple.

Our medium has the following composition: proteose peptone #3, 10 grams; dextrose, 5 grams; sodium chloride, 4 grams; agar agar, 4 grams; and distilled water, 1,000 ml. Other peptones and modifications may possibly be used without materially affecting the results.

The reaction of the medium is adjusted to pH 7.6–7.8 before sterilization and before the addition of the drug solution. The medium is dispensed in 90-ml. quantities, and to obtain approximately 10 mg. per cent concentration of the drug in the medium, 10 ml. of 100 mg. per cent solution of the drug are added. After mixing, the medium containing the drug is dispensed in 10-ml. amounts into 16-mm. culture tubes before sterilization in the autoclave at 15 pounds pressure for 15 minutes. The final concentration of the drug can be varied by addition of the proper amount of drug and making up to 100 ml. with more basic medium.

While our experience in testing sulfonamide resistance or susceptibility has been mostly with hemolytic streptococci which grow well in the basic medium, it has been observed that other bacteria grow well also. Growth of members of the *Neisseria* is supported, although the results obtained in testing the susceptibility of the gonococci to sulfonamides with this medium have not been as satisfactory as with streptococci. From results obtained it is obvious that the medium is satisfactory for the cultivation of the majority of pathogenic bacteria. Our experience with this medium has shown that there is little or no antagonistic effect upon the sulfonamides.

Penicillin has been added to this medium in various amounts, and the susceptibility of streptococci and other bacteria to this antibiotic has been determined by this method with satisfactory results.

In the earlier tests with this medium the sulfonamides were prepared in sterile, distilled water and added to the melted medium. No contamination resulted, but addition of the drug solution to tubes required accurate measurements of both medium and drug. This is time-consuming and tedious and tends to introduce errors. It was decided to add the drug solutions to the medium before sterilization and note the effect. Parallel tests using the same medium and drug concentrations sterilized by autoclaving with the medium or added without sterilizing showed no appreciable difference on the bacteriostatic effect of the drug. The concentration of the drug in the medium was determined before and after sterilization by the method of Bratton and Marshall (*1*) for blood sulfonamide determinations. There was slight, but not appreciable, reduction of sulfonamide concentration after sterilization (Table 1).

TABLE 1

Drug	In water	Not autoclaved	Autoclaved in medium
	mg. per cent	mg. per cent	mg. per cent
Sulfanilamide	11.4	9.4	9.2
Sodium sulfathiazole ..	11.7	9.1	9.0
Sodium sulfapyridine ..	11.0	9.1	8.9

The first dilution of drug is prepared in sterile, distilled water by placing 100 mg. of the drug into 100-ml. water. The next dilution is made by adding 1.0 ml. of the first dilution to 9.0 ml. of distilled water or 9.0 ml. of the medium. It will be noted (Table 1) that there is an immediate drop in sulfonamide concentration when the drug is added to the medium. Autoclaving the drug with the medium brings about another slight drop in drug concentration. These decreases may be allowed for by the addition of slight excess of drug when added to the medium. There is no further decrease in concentration upon incubation, and the medium may be stored for two weeks, or longer, without decrease in drug concentration.

It will be seen from a glance at Table 2 that there

is no difference in the results obtained with media sterilized with and without the drug.

The control (+) contained approximately 20 colonies in each case. Where growth occurred in the presence

TABLE 2

Drugs added after medium was sterilized	Concentration of drug mg. per cent	Cultures 089 MA		N 360 C		71	147
		24	48	24	48	24	48
Sulfanilamide	9.4	+	+	–	–	+	+
Sodium sulfathiazole ..	9.1	–	–	–	–	–	–
Sodium sulfapyridine ..	9.1	–	–	–	–	–	–
Drugs sterilized in autoclave with medium							
Sulfanilamide	9.2	+	+	–	–	+	+
Sodium sulfathiazole ..	9.0	–	–	–	–	–	–
Sodium sulfapyridine ..	8.9	–	–	–	–	–	1 colony
Control	None	+	+	+	+	+	+

of sulfonamide (Table 2) there were fewer than 20 colonies but sufficient growth to indicate resistance to 9.2 and 9.4 mg. per cent of drug. One colony appeared in medium containing 8.9 mg. per cent of sodium sulfapyridine after 48 hours' incubation.

TABLE 3

Culture	Control		Sulfanilamide		Sodium sulfathiazole		Sodium sulfapyridine	
	24	48	24	48	24	48	24	48
1296	+	20 colonies	0	0	0	0	0	0
156	?	10 colonies	0	0	0	0	0	0
154	+	20 colonies	0	0	0	0	0	0
153	+	34 colonies	0	0	0	0	0	0
360	+	100 colonies	0	0	0	0	0	0
1172	?	2 colonies	?	6 colonies	0	0	0	6 colonies
1467	+	>100 colonies	+	>100 colonies	+	>100 colonies	+	>100 colonies
1060	+	35 colonies		30 colonies	0	0	?	20 colonies
147	+	+	0	0	0	0	0	0
089 MA	+	+	0	0	0	0	0	0

Culture 147 is, therefore, somewhat resistant to this drug.

The convenience of preparing the medium containing the drug makes routine testing of sulfonamide resistance or susceptibility a simple matter, once the medium is prepared, amounting to no more than the inoculation of one more tube and a control tube. A series of tubes containing graduated amounts of the desired sulfonamide may be prepared. For this study a concentration of approximately 10 mg. per cent in the medium has been used and has been satisfactory in most cases for determination of drug resistance or susceptibility. Other concentrations of the drug have been used to determine degree of resistance.

Table 3 shows the results of a typical day of testing with hemolytic streptococcus cultures using 10 mg. per cent concentration of the sulfonamide indicated.

Usually results can be read after 24-hour incubation. Occasionally resistance to sulfonamide activity can be seen after 48-hour incubation and would be missed if read only in 24 hours. For this reason our results are read after 24- *and* 48-hour incubation.

Inoculation of the media for these tests is done by taking a loop of an 18- to 24-hour broth culture into a tube of tryptose-phosphate broth. One loop of this dilution into the melted (and cooled) semisolid medium containing the drug will generally give 10 to 20 colonies in the control tube. The loop that we have used measures 5.0 mm. in outside diameter and is made from 28-gauge platinum wire. The loop is flamed between each inoculation to remove traces of drug and agar and to keep the size of the inoculum as constant as possible.

While our particular interest in this problem has been to devise a simple and convenient method for testing sulfonamide resistance and susceptibility of hemolytic streptococci, the method can probably be used for testing other groups of organisms.

References

1. Bratton, A. C., and Marshall, E. K., Jr. *J. biol. Chem.*, 1939, **128**, 537.
2. Wilson, Armin T. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 130.

About the Chemical Nature of Syphilis Antigen

E. Fischer and F. Velasco

Cathedra of Anatomy and Physiology, Universidad de Chile, Santiago

In 1942 M. C. Pangborn (*3*) described a method of purifying the syphilis antigen of alcoholic beef heart extract by precipitating the phosphatides with $CdCl_2$, suspending the precipitate in petroleum ether, and eliminating the lecithin $CdCl_2$ complex by extractions with 80 per cent alcohol. The remaining solution contained cephalin $CdCl_2$ complex and antigen. After eliminating Cd by NH_3 and cephalin by precipitations with alcohol, the antigen was further purified until a substance which Pangborn claimed to be the pure syphilis antigen was obtained. According