DETERMINING THE GERMICIDAL EFFICIENCY OF DISINFECTANTS*

GEORGE F. REDDISH, PH.D.

Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.

OUR so-called standard methods of testing the germicidal efficiency of disinfectants have never been entirely satisfactory. Within recent years, especially, most workers in this field of bacteriology have agreed that a "phenol coefficient," determined by either the Rideal-Walker or the Hygienic Laboratory method, is nothing more than an indication of the germicidal efficiency of specific substances against the test organism, *Bacillus typhosus,* under the specified conditions. Various laboratories have in many cases deviated from the published methods in testing disinfectants, but have indicated that their results were obtained by one or the other of the accepted procedures. Individual workers have not been satisfied with every detail of these methods and have consequently made changes which they have considered justifiable. This has in most cases been responsible for lack of uniformity in results obtained. However, different laboratories attempting to make comparative tests on specified disinfectants by means of the Rideal-Walker and Hygienic Laboratory methods found considerable variations in results under the best conditions.

The Committee on Standard Methods of Examining Disinfectants of the American Public Health Association, seeing the need for a more accurate method for testing disinfectants,[1] made a number of changes in the existing Hygienic Laboratory Method, adding the refinements needed to increase its accuracy. This method was accepted and published as the "A.P.H.A. standard phenol coefficient." This method while fairly accurate was far too complicated, cumbersome and expensive to be practicable in routine testing of disinfectants and was never generally used. The slight increase in accuracy of the results obtained by this method over that of those obtained by the Rideal-Walker and Hygienic Laboratory methods did not justify the expenditure of time and effort necessary to obtain the information desired. After all, the knowledge gained by the use of this elaborate procedure was nothing more than a fairly accurate estimation of the strengths of disinfectants necessary to kill one pathogen, *B. typhosus,* as compared to the strength of phenol necessary to accomplish the same result under the conditions of the test. The A.P.H.A. method was later abandoned by the Committee on Standard Methods.

However, one important suggestion made was that future work on disinfectants should include the use of disease-producing types of bacteria other than *B. typhosus,* and should be in the nature of phenol coefficient tests. It is generally conceded that further complication of the method of testing disinfectants would not be favorably accepted by those who have to do the actual work; in fact, a simplification of procedure would be most welcome. The writer has already suggested[2] that disinfectants be tested against representatives of the most important groups of pathogens in the simplest manner possible. That is, the dilutions of disinfect-

* Report of the Referee on Standardization of Disinfectants read before the Laboratory Section of the American Public Health Association at the Fifty-fourth Annual Meeting at St. Louis, Mo., October 19, 1925.

ants which will kill various pathogens should be determined without any comparison to phenol as a standard. A phenol control should always be used, of course, as a check on the resistance of the culture. Although there may not be an accurate correlation between the resistance of organisms to phenol and the irresistance to other disinfectants, for the present we are justified in judging the resistance of test organisms by their resistance to phenol. Such control should be included in each test when the various pathogens are used. In this test there would be no figures showing the strength of disinfectant as compared to phenol, but simply information as to the efficiency of certain dilutions of disinfectant against pathogens met in practice. The phenol control would be used solely as a check on the resistance of each particular culture used at the time the test was made.

The seven pathogens representative of their respective groups, already suggested[2] as test organisms for determining the dilution in which disinfectants will be effective against them, are:

B. typhosus (representative of the Gram negative, non-sporing bacilli)

Staph. aureus (representative of the suppurative group, and also Gram positive cocci)

B. diphtheriae (representative of granular Gram positive group, and diphtheroids)

B. tuberculosis (representative of the acid-fast group)

Dip. pneumoniae (representative of the encapsulated, Gram positive cocci)

Strep. hemolyticus (representative of the septicemic pathogens, scarlet fever organisms, erysipelas, etc., Gram positive chain-forming cocci)

B. pestis (representative of the hemorrhagic-septicemic group)

A preliminary attempt was also made to establish a standard for *Staph. aureus* strains for use in such tests, after studying the resistance of 25 strains of this organism to phenol. The results of this study suggested that the strain of *Staph. aureus* to be used in disinfectant testing should have a certain resistance to phenol. It should not be killed by 1–70 phenol in 10 minutes, nor by 1–80 phenol in 15 minutes; it may or may not be killed by 1–60 phenol in 5 minutes, this dilution being on the border line. When a disinfectant is recommended for a purpose in which the killing of suppurative organisms would be expected, and certainly desired, such as hand disinfection, treatment of cuts, wounds and sores, and hospital disinfection, it should be tested against a vigorous resistant strain of *Staph. aureus,* one which comes within the limits specified in the standard suggested.

Certain factors under practical conditions may be hard to include in laboratory tests—conditions that may interfere with the efficiency of the disinfectants. It is well, therefore, to allow for a margin of safety and use enough culture to allow for the maximum of organisms and a certain amount of organic matter. A dilution of 1 part culture to 10 parts disinfectant seems adequate. In the Rideal-Walker technique, 0.5 c.c. of culture to 5.0 c.c. of disinfectant dilution is used. This proportion has been used also in the work on *Staph. aureus* reported previously,[2] and in subsequent work to be reported later. The results obtained with broth cultures filtered through filter paper were the same as those obtained when unfiltered cultures were used. The tests were carried out at 20°C. Test cultures were made in beef extract broth, pH 6.5 to 6.8 and 24-hour cultures were used (after transferring daily for 5 days). Transfers were made into 10 c.c. of similar broth, and incubated at 37°C for 48 hours. Under such conditions *Staph. aureus* did not vary more in its resistance to a coal-tar compound of definitely known composition than did *B. typhosus.* This disinfectant was tested at various intervals over a period of 7 weeks, both *B. typhosus* and *Staph. aureus* being used under the same conditions. The dilutions required to kill the two organisms differed greatly, but varied as much for one as

for the other, and this only slightly. We can say, then, that as a test organism *Staph. aureus* is as reliable as *B. typhosus* under specified conditions.

Under proper conditions *B. typhosus* is a very good test organism, and there is no reason for abandoning it. We are interested here, not in substituting a test organism for *B. typhosus,* but in adding other pathogens as test organisms. In order to determine accurately the efficiency of a disinfectant against pathogens, various organisms, including *B. typhosus,* should be used. Manufacturers of disinfectants, before recommending dilutions for their products, should have definite information regarding strengths necessary to kill pathogens representative of the most important groups. From a regulatory standpoint, it would only be necessary to test disinfectants against these pathogens in the dilutions recommended by the manufacturer. If a dilution of 1–100 is specified for general disinfection, for the body, wounds, etc., this dilution would then be tested against the proper pathogens for, say, 5 and 15 minutes.

Because of the known specificity of many disinfectants, it is desirable that specific information be had concerning the efficiency of various substances against all the pathogens that the physician might wish to kill. For example, it is known that pine oil, which is very effective against *B. typhosus* and certain other pathogens, is of no value against *Staph. aureus.* It is possible that a similar specificity may be found for other disinfectants as well. For substances used for wound disinfection, the tubercle bacillus would not be used as a test organism, but those disinfectants to be used in tuberculosis sanatoriums should be tested and found efficient against this organism. Disinfectants for use around the ordinary hospital should kill the ordinary Gram negative nonsporing organisms and the streptococci, staphylococci, pneumococci, and diphtheria bacillus. Also those substances used for general household disinfectants for use on cuts, wounds and sores should kill *Staph. aureus* in the dilutions specified.

Naturally in such tests the resistance of the organisms employed must be taken into consideration, or rather, only such strains of these organisms as are shown to have high natural resistance should be used. In the Rideal-Walker test it is specified that the culture of *B. typhosus* used should be discarded if it is killed by 1–110 in 2.5 minutes, or if it is not killed by 1–90 dilution in 7.5 minutes. A somewhat similar standard has been suggested for *Staph. aureus* whereby the resistance of the strain used may be gauged by selecting only those which come within the limits of this standard. Some such standard must be established for every organism to be used in disinfectant testing. For this purpose it will be necessary to test a number of new isolations of the organisms selected against a known, constant germicidal substance, such as phenol. From my study of ten new strains of *Streptococcus hemolyticus* I am prepared to offer a tentative standard for these streptococci. Only ten strains were used: twice this number should be studied before a definite criterion is accepted.

Streptococcus hemolyticus grows very readily and abundantly in glucose broth of pH 7.2 to 7.4. It will grow well in plain broth of the same pH also, but less luxuriantly. A few strains were grown in dextrose broth for 5 days, transferring at 24-hour intervals, and tested for their resistance to phenol, using a technique resembling in some respects both the Rideal-Walker and the Hygienic Laboratory tests. At the same time similar tests were made with cultures grown in the plain broth, pH 7.2 to 7.4. Owing to the formation of acid from glucose, the organisms in glucose broth were killed by a much more dilute solution of phenol than those in plain broth. A culture grown in glucose broth was killed by 1–110 phenol in 5 minutes, but when grown in plain broth of pH 7.2 it was not killed by 1–70 phenol in 5 minutes, nor by 1–80 phenol in 10 minutes.

Therefore, in the tests made with streptococci, the following technique was employed: Isolations were made from fresh pathologic material, transferred into glucose broth in order to obtain vigorous growth, inoculated into plain broth, pH 7.2 to 7.4, and cultured in this medium for 5 days at 37°C, transferring at 24-hour intervals. On the 5th day, 0.5 c.c. of the plain broth culture was added to 5 c.c. portions of various dilutions of phenol held at 20°C for 5, 10 and 15 minutes, at the end of which time transfers were made into 10 c.c. of *glucose broth* and incubated for 48 hours at 37°C. The material was transferred into glucose broth because it was found that when only a few living organisms, weakened by exposure to phenol, were added to plain broth they would not grow, whereas conditions in glucose broth were so favorable that these weakened organisms grew.

Following this technique, ten strains were tested against the following dilutions of phenol: 1–70, 1–80, 1–90, 1–100 and 1–110. The growth in the subculture tubes was confirmed. The following results were obtained: 5, or one-half of the strains were killed by 1–70 phenol in 5 minutes, 8 of them by 1–70 phenol in 10 minutes, and all of them by 1–70 phenol in 15 minutes. All strains survived exposure to 1–80 phenol for 5 minutes, 7 survived 1–80 for 10 minutes, and all were killed by 1–80 for 15 minutes. None of the strains was killed by 1–90 in 5 and 10 minutes, and only one by 1–90 in 15 minutes. From these data it can be safely concluded that strains of *Strep. hemolyticus* to be used in testing disinfectants should not be killed by 1–90 phenol in 15 minutes nor by 1–80 phenol in 5 minutes under the conditions specified, or strains should not be killed by 1–90 phenol in 15 minutes, and should be killed by 1–70 in 10 minutes.

Just how long these strains will retain their resistance to phenol or any other disinfectant when carried in artificial media is not known at present. It may be assumed that they will not continue resistant so long as *B. typhosus* and *Staph. aureus* under the same conditions. In a hormone agar or even in egg-meat medium, however, they may be expected to retain their vigor and resistance for some time. Even if they should not continue resistant for long periods of time in artificial media, it would be simple enough, and convenient in the laboratories where disinfectants are tested, to obtain new isolations from time to time. The value of the information obtained by the use of a streptococcus in testing disinfectants justifies any inconvenience which might be entailed in obtaining new strains at various intervals.

Although there are many reasons why the phenol coefficient test as now used might well be replaced by the scheme here outlined, it seems advisable to continue its use in some form until the details of the new plan are perfected. Some comparative figure on *B. typhosus* found by a simplified test should probably be retained until this scheme has become firmly established.

REFERENCES

1. Report of the Committee on Standard Methods of Examining Disinfectants. *A. J. P. H.*, 7, 506–521 (July), 1918.

2. Reddish, G. F. The Resistance to Phenol of *Staphylococcus aureus*. *A. J. P. H.*, 15, 534–538 (June), 1925.