

## Methods of Evaluating New Antiseptic Agents

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The evaluation of the antibacterial activity of new antiseptics has been and still is a major problem confronting bacteriologists. Many tests have been devised to determine the antiseptic qualities of various compounds. No single one of these tests, however, is without its limitations and deficiencies.

Consequently, in attempting intelligently to evaluate any agent, a testing program must be devised which includes as many different types of antiseptic tests as are feasible. Especially in the case of the comparatively new quaternary ammonium cationic compounds is the foregoing true. These compounds have been enjoying increasing popularity for use as antiseptics. Their antibacterial activity in high dilutions has brought them into demand in industries where there is a need for economical and efficient chemical sterilizing agents.

The question now arises, what various tests should be done in order to determine the activity of an antiseptic? The answer to this, of course, depends upon the properties which you desire to determine in a given germicide. Recently, Salle and Catlin (3) made an excellent suggestion for the evaluation of germicides. They recommended that, in the evaluation of an antiseptic, tests should be carried out which would determine the following minimum properties of antiseptics: (1) the highest dilution of germicide capable of killing *Micrococcus pyogenes* var. *aureus*; (2) extent of bacteriostasis; (3) influence of organic matter; (4) speed of action; (5) penetrability; and (6) toxicity.

For this purpose the following tests were recommended: (1) modification of the FDA test for determining the killing dilution against *M. pyogenes* var. *aureus*; (2) bacteriostatic tests in 10 and 100 ml of FDA broth and thioglycollate medium; (3) similar tests in the presence of 10% serum; (4) tests for killing, in time periods of from 1 to 10 minutes; (5) penetration tests, using the FDA agar cup plate method; and (6) toxicity tests on embryonic chicken heart tissue.

According to Reddish (2), "Studies of this character give considerable information relative to the properties of antiseptics. In fact, a combination of these tests will give information from which the practical value of an antiseptic may be predicted, or at least they will reflect its potentialities. Furthermore, the methods employed are all standard procedures that have been used separately for some years . . . . Since each test shows certain specific properties of antiseptics, collective results give a correct and useful profile of the antiseptics tested."

In addition to these minimum properties, other characteristics may be present in a new compound and tests designed to evaluate these properties should be done.

For several years in our laboratories efforts have been directed toward the development of a general household antiseptic. The final formula contained a variety of pharmaceutical compounds along with the quaternary ammonium compound, di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride, as the antiseptic ingredient.

During the course of the development of the formula, antibacterial tests were chosen which unknowingly followed Salle's recommendations with some modifications.

The FDA Test for Liquid Antiseptics was used to determine the highest dilution of the antiseptic capable of killing *M. pyogenes* var. *aureus* in 10 but not 5 minutes. The inconsistencies that occur in phenol coefficient results on highly active quaternary ammonium compounds have been the subject of interest to investigators for several years. Inconstancy of the culture media, phospholipid content of peptones, mutation of the test organism, loop transfer method, agglutination of the bacteria, and bacteriostasis by the quaternary ammonium compounds have all been cited as being contributing factors to inconsistent end points in the test. Various modifications have been proposed to overcome some of these objections. Consequently, in our hands, the method was modified in that vigorous shaking prior to making each loop subculture of the medicant was done to reduce clumping and adherence of the bacteria to the side of the tube and a so-called T.A.T. broth, proposed by Armbruster and Ridenour (1), was used as a quaternary ammonium compound neutralizing subculture medium to overcome the bacteriostatic effect of the "quat." Only thru the use of these modifications and performance of the test many times were we able to eliminate "spotty" results and determine the highest dilution being bactericidal for *M. pyogenes* var. *aureus* and a variety of other organisms in 10 but not 5 minutes in the presence and absence of 10% serum at 20 and 37°C. Because of its relative simplicity and reproducibility in our hands, the method is now used in our laboratory as the primary bacteriological control test on newly manufactured lots of the antiseptic.

The Kolmer Bacteriostatic Test was employed to determine the extent of bacteriostasis. The test is extremely simple and yields sharply defined results. It determines the highest dilution of a disinfectant capable of restraining the growth of the test organism for a stated period of time and is of particular value for comparing the antiseptic properties of various chemical agents. At the end of a given period of time, the tubes may be subcultured onto a solid medium to determine whether the organisms have been killed or merely restrained. In this manner, a bactericidal test is also conducted.

To determine the speed of action, a so-called "percentage-kill" test was done. Briefly, this involved a mixing of the test organism and antiseptic in a special mixing apparatus. At various intervals of from 1 to 10 minutes, samples were removed and pour plates made from which the number of surviving bacteria was determined. The number of surviving organisms was then plotted against time of exposure to

the antiseptic to determine the number of organisms killed per unit of time.

Although agar-plate or cylinder-plate methods of testing have been widely used with chemotherapeutic agents, these methods of testing for bactericidal activity have not been found to be applicable to quaternary ammonium compound testing, possibly due to the inability of ionic aggregates to pass through the agar network or due to physical adsorption of the cationic compounds on the agar. However, the methods are useful in that a comparative penetrability of various quaternary ammonium compounds through agar may be determined. Therefore, the penetrability of the antiseptic, not the bactericidal activity, was determined by the Filter Paper Disc Method.

Chronic and acute toxicity studies carried out by another department of the laboratory by standard pharmacological methods, and tests made on exposed areas of skin which revealed no demonstrable cutaneous reactions indicated the low order of toxicity of the formula.

Consequently, from the results of these tests, we knew the highest dilution of the formula capable of killing *M. pyogenes* var. *aureus* and other organisms in 10 but not 5 minutes; we knew the extent of bacteriostasis of the antiseptic; we knew the speed of action; we knew the effect of organic matter and temperature upon the activity of the germicide; we knew its penetrability on agar as compared to similar compounds; and finally we knew that it was relatively non-toxic.

Inasmuch as each of these tests measures only one specific property of antiseptics, the results, when considered individually, tell little or nothing concerning the activity of an antiseptic. In fact, the evaluation of an antiseptic on the basis of any single one of these tests alone may easily prove to be an embarrassing and costly error. It is only when considered collectively that the results assume a significant meaning insofar as the overall activity of the newly-developed antiseptic is concerned.

With these basic characteristics determined, additional properties that the newly-developed antiseptic may be suspected of possessing can be evaluated. For our specific antiseptic, there were several. It was desirable to know the effect of the formula upon various contaminated objects such as wood, cloth, instruments, skin and the like.

Since the general trend in antiseptic testing today favors tests which more accurately simulate conditions of actual use of the germicide, laborious and time-consuming tests were employed to evaluate these properties under as closely simulated conditions of use as laboratory facilities would permit.

The results of these tests indicated that this specific antiseptic formula was highly effective on cloth, wood, instruments and skin. It was also demonstrated that a considerable amount of residual antibacterial activity was bestowed by the antiseptic onto cloth and skin and presumably onto other surfaces. Various other types of *in vitro* and *in vivo* tests were also performed which gave confirmatory or incidental information relative to the activity of the antiseptic.

Consequently, then, the collective results of all these various tests gave us a comparatively clear picture of the antibacterial activity of the antiseptic formula developed in our laboratory—a picture from which we could intelligently predict the practical value of the germicide—a picture which no one single antiseptic test could give us, and the clinical use of this antiseptic thus far has borne out our predictions extremely well.

#### Literature Cited

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