Studies on the Efficiencies of Disinfectants for Use on Inanimate Objects

I. Relative Activities on a Stainless Steel Surface Using a New Performance Test Method[1]

R. L. Stedman, E. Kravitz, and H. Bell

Industrial Test Laboratory, Philadelphia Naval Shipyard, Naval Base, Philadelphia, Pennsylvania

Received for publication November 20, 1953

After many years of investigation on the theoretical and practical aspects of disinfection, the proper choice of a disinfectant for use on inanimate objects still remains a matter of great controversy (Klarmann and Wright, 1946, 1947 and 1948; Dubois, 1947; James, 1947; Rahn and Van Eseltine, 1947; Klimek and Umbreit, 1948; Stuart, Bogusky and Friedl, 1950; Klarmann, Wright, and Shternov, 1953). Much of the controversy arises from the innate complexity of the disinfecting procedure as performed in practice. The degree of cleanliness of the surface, the nature of the cleansing agent (if one is used), the composition of the surface, the technic used in applying the disinfectant, and the particular organisms to be encountered are among the more important factors which influence the proper choice of a disinfectant.

A large number of test methods have been proposed which incorporate steps designed to study the effect of these factors on germicidal agents. As part of a large project involving the study of disinfection, this laboratory has developed a performance test method intended to simulate actual-use conditions. The method is capable of being modified to incorporate many of the environmental factors affecting disinfection. In this presentation, the method has been used to study the antimicrobial efficiencies of several types of disinfectants on a stainless steel surface.

Experimental Method

Development of Method

Preliminary studies were directed toward developing a method which incorporated steps identical with those used in practice. Initially, the technic consisted of contaminating a large stainless steel surface, mopping the area with the disinfectant, permitting the disinfectant to act for a period of time, and recoverng the survivors. However, it was found that quantitative recovery of organisms from large surface areas using a standardized swabbing procedure could not be consistently attained. It was then decided to cut up the surface into one-inch squares,[2] seed each, disinfect each by a standardized procedure, and recover the survivors from each square. It was found that quantitative recovery of organisms from these squares could be achieved by either one of two methods: plating the squares directly into agar medium; or rinsing the squares in 20 ml of diluent and plating the diluent. The swab method was shown to be inferior to either of these procedures. Ultimately, the rinsing technic was chosen since it permitted differential culturing of different species from a single square.

Since the test method was to consist of disinfecting dried films of organisms, test species were selected which were representative of broad pathogenic types and were relatively resistant to drying. *Micrococcus pyogenes* var. *aureus* was found to possess comparatively high resistance to drying and was chosen as a typical, pyogenic, gram positive coccus; *Salmonella schottmuelleri* was the most resistant of the enteric species tested; *Trichophyton interdigitale* possessed adequate resistance to drying and was selected as a typical dermatophytic fungus. The question of the statistical variation in the numbers of surviving cells recovered from films of organisms on replicate squares was subsequently studied and found to be satisfactory.

Later, it was found that the survival rate of the organisms in dried films varied significantly in experiments conducted from day to day. In the majority of tests, inoculation and drying of squares as outlined below gave 100,000 to 2,000,000 *Micrococcus pyogenes* var. *aureus* cells per square and only tests giving this range of survivors from control squares were included in the data presented here. With the fungus, the range was 5,000 to 25,000 spores per square with the majority of tests being in the upper range. The gram negative organism varied more widely; in the tests reported below, all control squares gave 50,000 to 2,000,000

[1] The views expressed herein are those of the authors and not necessarily of the Department of the Navy.

[2] Stainless steel conforming to Specification MIL-S-854, Class 1, 16 gauge, condition "a," finish no. 4.

Salmonella schottmuelleri cells per square. During the course of the study, however, much lower numbers of *Salmonella schottmuelleri* cells were occasionally recovered, and the results of these tests were discarded. Available evidence indicated that variation of the inoculum over the indicated ranges did not affect the activities of the disinfectants. At present, work is being continued on ways of overcoming the erratic drying effect with *Salmonella schottmuelleri*.

Other factors which might affect the significance of this test method have been studied, but are too numerous to mention here. None of the factors investigated has been found to affect the validity of the procedure.

Details of the Method

As previously mentioned, the method consists of drying organisms on the surfaces of 1-inch stainless steel squares, applying the disinfectant to the surfaces for 10 minutes, and recovering the survivors. The method differs from other carrier-type technics in that a mixture of organisms is used, the squares are not immersed in the disinfectant, and the organisms are recovered quantitatively, rather than qualitatively. Details of the method are as follows:

M. pyogenes var. *aureus* 209 and *S. schottmuelleri* (ATCC #9282) are grown independently in nutrient broth consisting of 1.0 per cent Difco peptone, 0.5 per cent NaCl and 0.5 per cent Difco beef extract for 24 hours at 37 C. *T. interdigitale* (ATCC 9533) is grown on Difco Sabouraud maltose agar for 12 to 14 days at 25 to 30 C; the growth is scraped from the slant and suspended in water; the suspension is filtered through absorbent cotton and adjusted to a microscopic count of about 5,000,000 conidia per ml. Inoculum for the test is made by mixing 1.0 ml of each of the bacterial cultures with 1.0 ml of the spore suspension and 0.6 ml sterile broth. If organic matter is desired, it is substituted for the sterile broth; using normal horse serum, a concentration of approximately 10 per cent serum is thus obtained on subsequent addition of the disinfectant to each square.

A 0.025-ml aliquot of the inoculum is added to each square and spread uniformly with a platinum wire.[3] All squares are air-dried in an incubator at 20 C. The time for complete drying of these squares is approximately 20 to 30 minutes and all squares are used immediately after complete drying.

Various concentrations of disinfectant are made up in distilled water, 0.04 ml of each concentration added to each of a pair of dry, seeded squares, and the disinfectant and organisms mixed thoroughly over the surface using a platinum wire with a curve instead of a loop at the end. This quantity of disinfectant is the amount found by Klarmann and co-workers to be deposited on a unit floor area during a standardized mopping operation (Klarmann, Wright and Shternov, 1953). All disinfectant levels are tested in duplicate, using sterile water instead of disinfectant on one pair of squares as control.

The disinfectant dilutions are permitted to act for 10 minutes. Each square is then dropped into 20 ml of diluent, scraped with a sterile policeman and shaken. For testing phenolics and cresylics, distilled water is used as a diluent; for testing quaternary ammonium compounds, 0.07 per cent azolectin in aqueous 0.5 per cent Tween 80 is the diluent; for testing chlorine formulations, 0.5 per cent sodium thiosulfate is employed. Aliquots of the diluent are then plated in each of the following media:

Penicillin nutrient agar medium: Nutrient broth plus 2 per cent agar and 0.3 units crystalline penicillin G (potassium salt) per ml of medium.

Polymyxin agar medium: Nutrient broth plus 2 per cent agar and 150 units polymyxin B sulfate per ml of medium.

Terramycin agar medium: Nutrient broth plus 2 per cent agar and 4 micrograms Terramycin hydrochloride per ml of medium.

To prevent possible bacteriostasis in plating survivors of quaternary ammonium disinfectants, the same concentrations of azolectin and Tween 80 as used in the diluent are added to the nutrient agar. The inhibition levels of the antibiotics remain unchanged on the addition of the quaternary inhibitor. The penicillin and polymyxin plates are incubated at 37 C for 48 hours and the colonies of surviving *S. schottmuelleri* and *M. pyogenes* var. *aureus* cells in the respective media are counted. Counts of fungal survivors are made on the Terramycin agar plates after incubation at 25 to 30 C for 4 to 5 days, all bacteria being inhibited by the level of Terramycin used.

The average percentage reduction for each concentration of disinfectant is calculated for each organism, and the results are plotted as survival curves. The following disinfectants have been tested on a stainless steel surface by this method:

A pure quaternary ammonium salt of the structure, di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride (designated "Quat A").

A mixture of quaternary ammonium salts in a liquid concentrate. The active ingredients are alkyl (C_8–C_{18}) dimethyl 3,4-dichloro benzyl ammonium chloride and alkenyl (C_{16}–C_{20}) dimethyl ethyl ammonium bromide (designated "Quat B").

A detergent-sanitizer mixture consisting of 10 per cent of the above "Quat A" blended with a nonionic detergent and inorganic salts (designated "Detergent-Sanitizer").

A cresylic product containing a mixture of soap and cresylic acids (designated "Cresylic").

[3] A 0.2-ml Kahn pipette is used to measure the inoculum.

A synthetic phenolic formulation containing *o*-phenyl phenol and sodium ricinoleate as active ingredients (designated "Phenolic A").

A synthetic phenolic product of the emulsifiable type containing a mixture of 4-chloro-2-phenyl phenol, 6-chloro-2-phenyl phenol and anhydrous potassium castor soap as active ingredients (designated "Phenolic B").

A dry inorganic chlorine formulation containing 3.25 per cent sodium hypochlorite (designated "Chlorine Type"). The product is described as a stable, highly chlorinated tri-sodium phosphate formulation.

Results and Discussion

Selection of an End Point

Disinfection, by definition, implies 100 per cent kill of the specific infectious agents present. While the attainment of this degree of effectiveness in the usual methods of application may be questionable there can be no argument with the contention that a product sold to disinfect should be capable of effecting such a result if properly applied.

Because of the initial dilution of the survivors from squares by suspending the latter in 20 ml of diluent during the rinsing portion of the test method, it is not possible to determine a 100 per cent reduction end point unless the entire 20 ml are plated, an impractical procedure. The highest percentage reduction detectable will vary with the number of organisms on the control squares. When this number is 100,000 bacteria per square or higher (*M. pyogenes* var. *aureus* and *S. schottmuelleri*), a 99.99 per cent reduction can be determined if a 1.0-ml aliquot is plated; when the control squares give counts lower than 100,000 cells (*S. schottmuelleri*), the highest percentage reduction detectable drops progressively until a lower limit of 99.98 per cent is reached with control squares having 50,000 cells. For the fungus, the maximum reduction detectable is roughly one-tenth of the bacteria. Replicate runs on the products and the calculation of statistical means permit the acceptance of a 99.99 per cent reduction (bacteria) or a 99.9 per cent (fungus) reduction as the maximum reductions detectable in the tests reported here.

A second end point representing a significantly high percentage reduction has also been reported here to illustrate the profound effect of the dilution coefficient; this end point has been set at 99.9 per cent reduction for the bacteria and 99.0 per cent for the fungus.

The use of two end points gives a more accurate comparison of the germicidal activities of the products. Figure 1 illustrates clearly the marked effect of the dilution coefficient referred to above. In this figure, the percentage reductions with the "Cresylic" disinfectant and "Quat A" are plotted for the various dilutions tested against *M. pyogenes* var. *aureus* and *S. schottmuelleri*. With the "Cresylic" disinfectant, the slopes of the curves are much less than those encountered with "Quat A." This effect was evident with the quaternaries when tested in the absence of serum; in the presence of serum, however, the dilution coefficients of the various products were affected in different ways, again indicating that the use of a single end point will give an incomplete picture of the observed effects.

Accuracy of the Method

The accuracy of the method was found to be affected significantly by at least three factors: the relative size of the dilution coefficients of the disinfectants; the erratic disinfecting action exhibited by one class of products studied; and the range of variability encountered in replicate testing of identical concentrations.

Products having a small dilution coefficient exhibit a steep slope when the percentage reduction versus concentration is plotted logarithmically. When end points are selected from a steep slope a given degree of experimental variability on replicate testing will give a relatively high statistical deviation.

The second factor, erratic disinfecting action, was encountered with the quaternary products. Occasionally, squares disinfected with strong concentrations of the quaternaries showed a relatively large number of survivors even though subsequent weaker concentrations gave a higher percentage reduction. The use of replicate tests for calculating an average percentage reduction for each disinfectant level lessened the effect of these erratic findings. In only one case (indicated by footnote (||) in table 1) was there a single erratic finding of sufficient magnitude to produce a paradoxical picture, namely, the detergent-sanitizer product appeared to be more active in the presence of serum. Erratic findings occurred in only a few cases with the other types of products. Great care was taken to assure that technical error was not responsible for these "skips."

On replicate testing, the quaternary products exhibited a higher experimental variation than did the cresylic and phenolic types. The chlorine-type product gave a variability intermediate between the phenolic-cresylic types and the quaternary types.

Relative Efficiencies of the Disinfectant Types

Each disinfectant product was tested several times using the above method. The results are shown in table 1. For comparative purposes, all products except the quaternaries were tested by diluting the formulation as received; the quaternaries were diluted on the basis of active ingredient. In this table, however, all end points are based on active ingredients. With the phenolic and cresylic acid products used in this study, the soap plus the phenolic or cresylic acids were considered the active ingredients, as indicated by the labels on most of the disinfectants. Calculation of the

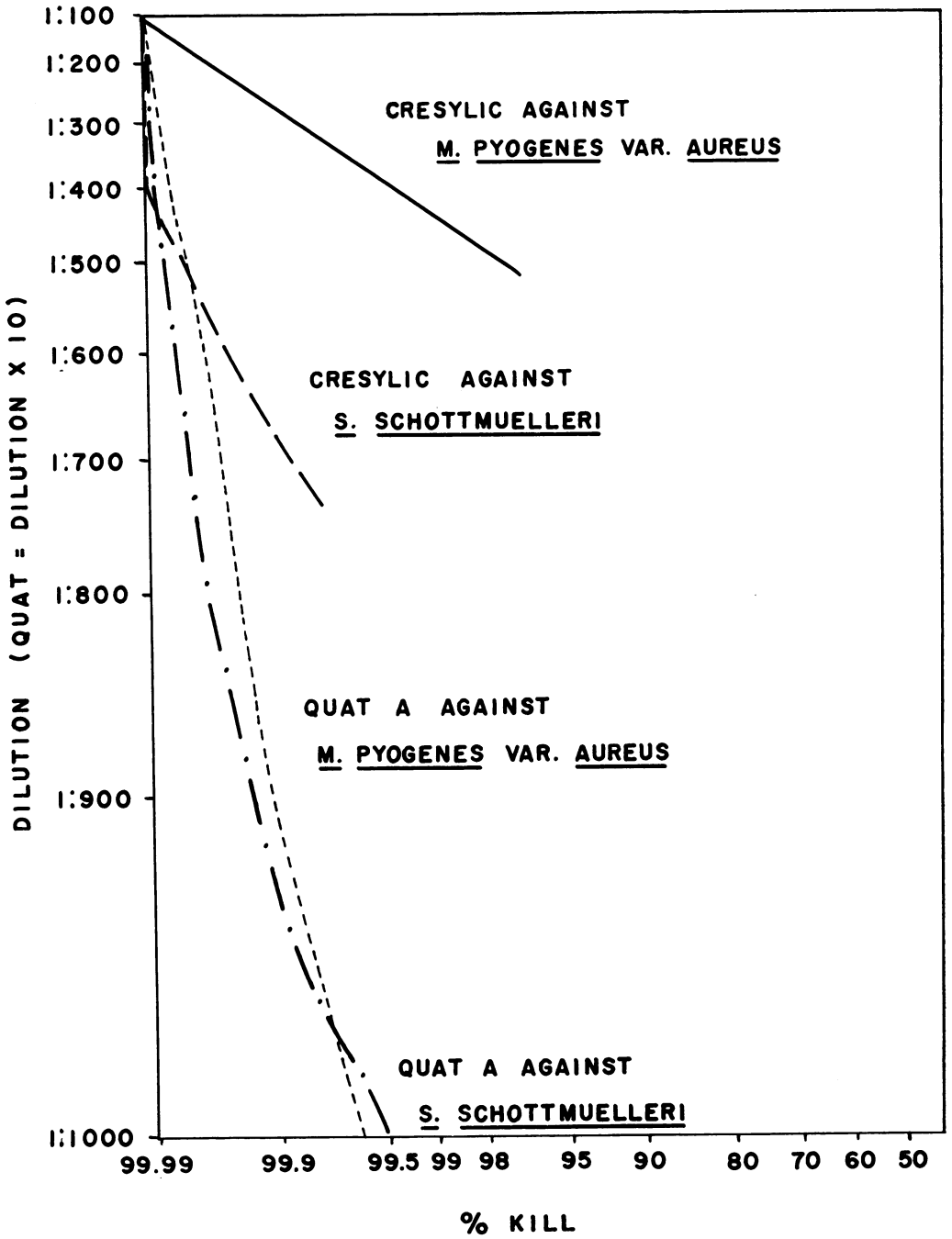

FIG. 1. Effect of dilution on the activities of a quaternary ammonium ("Quat A") and a cresylic disinfectant ("Cresylic"). Cresylic dilutions are of formulation; quaternary dilutions are of active ingredient.

active-ingredient dilutions from the formulation dilutions accounts for the uneven numbers listed in the table for phenolic, cresylic, and chlorine products.

In the absence of serum, the recommended use dilutions of the phenolic and cresylic products were adequate for disinfection in all cases. With the lower end point, a significant margin of safety was provided with these products. Under the same conditions, the recommended use dilutions of the quaternaries were not sufficient to achieve 99.99 per cent reduction of the bacteria but were satisfactory when 99.9 per cent reduction of the bacteria was measured. Except for the detergent-sanitizer, the quaternaries were markedly deficient in antifungal activity, requiring concentrations greater than 1 per cent for adequate disinfection; the formulation of "Quat A" into the "Detergent-Sanitizer" increased its antifungal activity (since these were performance tests, no attempt was made to control pH). The chlorine-type product was distinctly species specific. Against *M. pyogenes* var. *aureus*, relatively large concentrations of formulation were required for efficient kill. Against *S. schottmuelleri* and *T. interdigitale*, this product was effective, but concentrations stronger than those recommended for disinfection were required

TABLE 1. *Relative efficiencies of various types of disinfectants, using a new performance test method*

PRODUCT	RECOMMENDED USE DILUTION*	PERCENTAGE REDUCTION†	WITHOUT SERUM			WITH SERUM		
			Micrococcus pyogenes var. *aureus*	*Salmonella schottmuelleri*	*Trichophyton interdigitale*	*Micrococcus pyogenes* var. *aureus*	*Salmonella schottmuelleri*	*Trichophyton interdigitale*
Phenolic A	1:250	U	1:250	1:1500	1:250	1:130	1:250	1:130
		L	1:750	>1:1500	1:500	1:300	1:1000	1:250
Phenolic B	1:910	U	1:3600	1:5600	1:2700	1:910	1:910	1:730
		L	1:5000	>1:5600	1:4100	1:1100	1:2200	1:910
Cresylic	1:150	U	1:150	1:600	1:180	1:150	1:600	1:150
		L	1:380	>1:910	1:230	1:380	1:750	1:230
Chlorine type	1:5000 Available chlorine	U	1:310	1:3100	1:4600	<1:310	1:3100	1:4000
		L	1:620	1:3700	1:5200	1:310	1:3700	1:4600
Quaternary A	1:750‡	U	1:2000	1:1000	<1:100	1:500	1:1000	<1:100
	1:4000§	L	1:9500	1:9000	<1:100	1:2500	1:3000	<1:100
Quaternary B	1:2500¶	U	1:1000	1:1000	<1:100	1:1000	1:1000	<1:100
		L	1:4000	1:5000	<1:100	1:1500	1:1500	<1:100
Detergent sanitizer	1:5000	U	1:500	1:500	1:100	1:500	1:1000‖	1:100
		L	1:10,000	1:5000	1:500	1:3000	1:3000	1:400

* Based on active ingredients.
† U = 99.99 per cent kill (bacteria) and 99.9 per cent kill (fungus).
L = 99.9 per cent kill (bacteria) and 99.0 per cent kill (fungus).
‡ Recommended for use "against athlete's foot organisms."
§ Recommended for "general disinfectant use".
¶ Recommended for use where "contamination with infectious material has occurred".
‖ See Results and Discussion.

against the gram negative organism. The efficiency of this preparation against *T. interdigitale* was especially interesting, considering the widespread use of chlorine products in foot baths.

The effect of the dilution coefficient on the expression of results is well illustrated by the data obtained in the absence of serum. In the case of the quaternaries, from 4 to more than 20 times the amount of active ingredient was required to kill 99.99 per cent of the bacteria than was necessary to obtain 99.9 per cent reduction. With most of the other products, smaller increases in concentration raised the kill from 99.9 per cent to 99.99 per cent of the bacteria.

In the presence of serum, the pattern of relative activities changed. The bactericidal properties of the phenolics were reduced appreciably. Approximately two to six times more active ingredients were required to overcome the effect of the serum; approximately the same relative increase in concentration was necessary regardless of the end point used to measure the kill. The recommended use dilutions were sufficient to kill the test organisms at the lower end point; at the upper end point, the recommended use dilutions fell short in certain cases. Phenolic B was superior to Phenolic A in this respect.

The cresylic product required relatively slight increases in concentration to overcome the inhibiting effect of the serum and the recommended use dilution was effective in all cases.

The chlorine product retained its activity fairly well against two of the test organisms in the presence of serum; against *M. pyogenes* var. *aureus*, a significant increase in concentration was required to achieve comparable kill. Whether or not this effect is characteristic of all chlorine products is questionable since other types of chlorine preparations have not shown this effect when tested by other technics.

In the presence of serum, most of the quaternaries did not lose activity at the 99.99 per cent end point, but at higher dilutions giving the 99.9 per cent reduction, they lost activity in a manner similar to other type products. The recommended use dilutions of the quaternaries were insufficient to attain even a 99.9 per cent reduction of bacteria in the presence of serum.

Comments on the Relative Efficiencies of the Disinfectants

Under the use conditions employed above, all of the disinfectants perform adequately against bacteria if used in sufficient concentrations. However, not all of them can be regarded as highly effective when they are employed in the recommended manner. The chlorine product, for example, is recognized as an excellent dairy sanitizing agent when used at 200 ppm available chlo-

rine. When used as a disinfectant, however, the product is so species specific that an impractically high concentration of the formulation (10 per cent) is required for significant kill of *M. pyogenes* var. *aureus*. In addition, the question of stability may arise, although this material is claimed to possess relatively high stability. The quaternary products are dependable germicides against the bacteria when used in proper concentrations, but the present recommended use dilutions are too high. These use dilutions have been derived from the well-established but not universally applicable "20 times the phenol coefficient." Stuart, Ortenzio and Friedl (1953) have recently shown that this is not a constant to be employed with all disinfectants, but that its magnitude should vary inversely with the size of the phenol coefficient. Stuart, Bogusky and Friedl (1950) have also demonstrated this in another way, using a replicate testing procedure to show that the factor of 20 is not applicable to quaternaries. The findings reported here seem to confirm the contention of these workers. On the other hand, the low dilution coefficients of the quaternaries can be used with advantage if the appropriate use dilutions are initially employed. It is certainly advantageous for a disinfectant to kill high percentages of organisms when the germicide is diluted five- or tenfold beyond its recommended use dilution.

The occasional, erratic action of the quaternaries in high concentrations, permitting a relatively large number of survivors to be recovered, was disturbing. If such failures occur in practice, this may represent a serious shortcoming. When the number of erratic methodological experiences reported with these products is considered in relation to their mode of action and high affinity for protein, glass, and other materials, the germicides themselves appear erratic rather than the technician or the test method he uses.

The lack of fungicidal activity of the quaternary products was especially disappointing. A disinfectant should have significant antifungal activity at practical concentrations. In general, the phenolic and cresylic disinfectants appear to be fairly satisfactory in regard to spectrum and recommended use dilution. The cresylic product tested here was entirely adequate when employed in the recommended manner. The two phenolics varied somewhat from this pattern. One of these required a slight increase in use concentration to be entirely effective under all conditions; the other required a greater increase in recommended use concentration.

It should be emphasized that the above results and comments apply only to stainless steel surfaces contaminated with an arbitrary number of organisms and in the presence or absence of serum. Other pertinent factors have not been included here: the effect of humidity, the effect of natural waters of varying degrees of hardness, the presence of cleaner residues after a cleaning procedure has been performed, and the effect of other types of surfaces encountered in practice. Many of these factors are now being studied and the results will be reported at a later date.

Acknowledgment

Sincere appreciation is extended to Drs. L. Gershenfeld and L. S. Stuart for their helpful comments and suggestions in reviewing the manuscript.

Summary and Conclusions

A performance test method designed to simulate use conditions has been developed for testing inanimate-object disinfectants. The technic is fully adaptable for use in testing the effects of a number of environmental variables.

Using this method, the relative efficiencies of several representative types of disinfectants have been determined on stainless steel surfaces. All of the types possess adequate bactericidal activity if sufficient concentrations are employed. Increases in recommended use concentrations are recommended in various degrees for certain of the products.

The significance of these findings as well as details of the test procedure are discussed.

This tentative procedure is presented with the hope that others will try it in evaluating their products. The authors will appreciate comments.

REFERENCES

Dubois, A. S. 1947 Bacteriological evaluation of cationic germicides. Soap Sanit. Chemicals, **23** (5), 139–143.

James, L. H. 1947 Quaternaries vs. phenolics. Soap Sanit. Chemicals, **23** (11), 125–131, 152A.

Klarmann, E. G., and Wright, E. S. 1946 Quaternary ammonium germicides. Soap Sanit. Chemicals, **22** (8), 139–163.

Klarmann, E. G., and Wright, E. S. 1947 A note on the bacteriological evaluation of "cationics". Soap Sanit. Chemicals, **23** (7), 151–155.

Klarmann, E. G., and Wright, E. S. 1948 Concerning quaternaries. Soap Sanit. Chemicals, **24** (3), 155–159, 167.

Klarmann, E. G., Wright, E. S., and Shternov, V. 1953 Prolongation of the antibacterial potential of disinfected surfaces. Appl. Microbiol, **1**, 19–23.

Klimek, J. W., and Umbreit, L. E. 1948 On the germicidal behavior of a quaternary ammonium compound. Soap Sanit. Chemicals, **24** (1), 137–145, 159.

Rahn, O., and Van Eseltine, W. P. 1947 Quaternary ammonium compounds. Ann. Rev. Microbiol., **1**, 173–192.

Stuart, L. S., Bogusky, J., and Friedl, J. L. 1950 Difficulties encountered in testing quaternary germicides. Soap Sanit. Chemicals, **26** (2), 127–129, 145.

Stuart, L. S., Ortenzio, L. F., and Friedl, J. L. 1953 The phenol coefficient number as an index to the practical-use dilution for disinfecting. *Unpublished manuscript.*