A Method for Assessing the Sanitizing Efficiency of Quaternary Ammonium and Hypochlorite Products*

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THE effectiveness of hypochlorite solutions as sanitizing agents for the washed surfaces of food handling equipment is generally recognized, and these solutions are widely used for this purpose. More recently a number of "surface active" agents of the quaternary ammonium or cationic type have been advertised as substitutes for hypochlorites for this and other purposes. The present studies were undertaken in the hope of throwing some light upon the comparative efficiency of these two types of compound.

While the quaternary compounds show quite high values for phenol coefficient by the F.D.A. technique, the validity of this method has recently been called into serious question.[1, 2, 3] In any event, there is room for doubt as to whether such a technique is best suited to the task of evaluating the relative sanitizing efficiency of various solutions.

Some years ago, when faced with the problem of assessing the comparative germicidal speed of various chlorine compounds, a number of different techniques, including modifications of the F.D.A. method for the determination of phenol coefficient, were tried out. In an endeavor to approximate more closely the conditions under which a sanitizing solution has to do its work in actual practice, a glass slide method was finally developed which gave by far the most satisfactory results.[4] This method also permitted the use of very short periods of exposure to the germicidal solution, a point of considerable importance, since in many instances under practical conditions only a few seconds' contact is allowed. Preliminary tests showed that this glass slide technique, with slight modifications, could be used equally well with quaternary compounds, hence it was selected for the comparative tests to be reported in this paper.

DESCRIPTION OF GLASS SLIDE TECHNIQUE

In the glass slide technique, a 20–24 hour growth of the test organism on an appropriate agar medium is washed off and suspended in sterile distilled water. After filtration through a No. 1 Whatman paper the suspension is standardized to match the turbidity of a suspension of *Staphylococcus aureus* giving a plate count of 200,000,000 per ml. One ml. of suspension is then introduced into 60 ml. of a 1:10 dilution of sterile skim milk in a container of such dimensions that the depth of the liquid is 1½".

A previously sterilized slide is dipped into the seeded skim milk suspension so that the lower half of the slide is immersed. It is then carefully drained

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against the rim of the container for approximately 10 seconds and placed upright to drain upon a pad of sterile filter paper in a specially constructed draining can. Sufficient slides to run the desired number of tests on a given dilution of a product are prepared consecutively, following which the tests are carried out before the films of diluted skim milk have dried over more than 25 per cent of the area.

Previous to preparing the slides, 100 ml. portions of the solutions to be tested have been prepared with sterile distilled water. These are placed in 100 ml. beakers and brought to the desired temperature in a water bath. The slide first prepared is then drained of any excess liquid on the filter paper, dipped into the test solutions and gently agitated for the required period, which usually runs between 1 and 20 seconds in the present studies. It is then quickly removed, dipped momentarily into a beaker of tap water to rinse off the adhering germicide and minimize bacteriostatic action, shaken sharply once to remove excess rinse water, placed in a Petri dish and the plate immediately poured with an appropriate agar medium. The remaining slides in the set are similarly treated in correct sequence. After an appropriate period of incubation, the colony count of each plate showing less than 500 colonies is made and recorded.

In the sanitizing of equipment, utensils, etc., we are usually more interested in reducing the numbers of organisms below a certain arbitrarily established limit than in complete sterilization. It was therefore decided to take as the end point approximately 99.9 per cent destruction. To arrive at this figure, control plates are prepared at regular intervals during each day's run by dipping sterile slides into a second container of diluted skim milk in which the concentration of test organisms is only 1/60 to 1/600 of that of the original skim milk suspension. These slides are drained in the same manner as those treated with the germicide, then dipped momentarily in fresh tap water before plating. The average colony count on these control slides, multiplied by the appropriate factor, indicates the approximate number of organisms present on the regular slides before treatment. By dividing this figure by 1,000 a value is obtained representing the number of organisms remaining after 99.9 per cent have been destroyed.

ADVANTAGES OF GLASS SLIDE TECHNIQUE

Compared with the phenol coefficient technique, the most obvious advantage to the bacteriologist is the avoidance of the "skips" and "misses" which are so commonly encountered with that technique. Although irregularities are sometimes encountered, there is generally a steady drop in count with increasing period of exposure or increased concentration of germicide. Similarly, replicate runs conducted a week or more apart have generally indicated a satisfactory degree of reproducibility with this technique, as indicated by the data in Table 1. After all, it is reasonable to expect better agreement between replicate runs where 99.9 per cent destruction is taken as the end point than where the end point is taken as the killing of the last, most resistant, cell.

Another important advantage is that the glass slide technique more closely approximates the conditions under which a sanitizing agent has to work in actual practice. It measures the ability to kill organisms present on a surface in a film of organic matter, rather than freely suspended in the germicide. Furthermore, as a result of gentle agitation of the slide while immersed in the germicide, any superiority of a given product in detergent action has an opportunity to display itself and, by mechanical removal of the film and its accompanying

TABLE 1

Germicidal Potency of Various Sanitizing Agents Against Micrococcus candidus at 20° C. Replicate Determinations Conducted a Week Apart

Product	Concentration p.p.m.	Period of Exposure (Seconds) 1 a	1 b	1 c	5 a	5 b	5 c	10 a	10 b	10 c	20 a	20 b	20 c
Roccal	200	+	++	++	4	0	1	29	0	2	8	0	0
	100	+++	+++	+++	33	29	45	13	2	5	13	2	2
	50	+++	+++	+++	++	++	+	26	25	6	23	13	5
R-2-L	200	274	++	++	0	8	2	1	4	0	0	2	0
	100	+++	+++	+	59	12	0	2	7	0	3	1	0
	50	++++	+++	+++	++	+	+	27	24	37	15	15	18
Hyamine 1622	200	+	++	+	18	0	215	23	4	293	4	2	344
	100	+++	+++	++	34	23	39	13	46	24	7	25	37
	50	++++	+++	+++	+	+	+	32	163	29	14	96	16
Emulsept	200	++	++	+++	25	127	6	8	48	1	10	41	3
	100	+++	+++	+++	+	++	+	21	34	17	41	14	1
	50	++++	++++	+++	+++	+++	++	+	+	+	27	67	7
Dalglish	200	++++	++++	+++	+++	+++	+++	++	++	++	166	++	433
	100	++++	++++	+++	+++	+++	+++	++	++	++	484	++	+
	50	++++	++++	++++	+++	+++	+++	++	++	+++	169	+	+
Klenzade X4	200	++++	++++	++++	+++	+++	+++	++	++	++	981	++	++
	100	++++	++++	++++	+++	+++	+++	+++	++	++	+	++	++
	50	++++	++++	+++	+++	+++	+++	+++	++	++	++	++	++

a — 99.9% destruction leaves 91 colonies
b — " " " 114 "
c — " " " 62 "

bacteria, to reduce the number remaining on the slide.

Finally, attention should be drawn to the flexibility of this technique. The test organism, temperature of testing, period of exposure, concentration of germicide, plating medium, etc., may all be varied to suit the needs of the investigator.

EXPERIMENTAL

The products selected for comparative testing were representative of those on the market in Canada at that time, and included Roccal and R-2-L, both 10 per cent solutions of alkyl-dimethyl-benzyl-ammonium chloride, Hyamine 1622, di-isobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride in powder form, and Emulsept, a 10 per cent solution of N(acyl colamino formyl-methyl)pyridinium chloride. For comparison, two hypochlorites were selected. One, Dalglish Liquid Bleach, containing about 12 per cent available chlorine, is widely used throughout Eastern Canada. The other, Klenzade X4, is a newer product, said to be buffered to lower the pH and to render it more stable.

Time will not permit a detailed presentation of the results of these studies. They have included evaluation of the comparative germicidal efficiency against each of the test organisms at 20°C., while with three of these similar comparisons have also been made at 45°C. In addition, the effect of adjusting the pH has been studied with both *Staphylococcus aureus* and *Escherichia coli*. To give a general idea of our findings, a Table 2 has been constructed showing the periods of exposure necessary to effect 99.9 per cent destruction of each of the test organisms with one of the three concentrations of germicide tested. While this presentation tends to magnify certain small differences, as compared with the tables containing the complete data, it still serves to indicate certain important differences between the two types of sanitizing agent. For example, it clearly shows that against *S. aureus*, *Micrococcus candidus* and spores of *Bacillus panis*, the hypochlorites are definitely less effective than are the quaternaries. Against the vegetative cells of *B. panis* they are approximately equivalent, while against cheese starter organisms the hypochlorites are definitely more effective. The results from tests with flat sour organisms (21 hr. cultures) indicated destruction of >99 per cent of the cells by Roccal within 5–10 seconds, and by Dalglish hypochlorite within 10–20 seconds. How-

TABLE 2

Periods of Exposure (Seconds) Required to Destroy 99.9 Per cent of Test Organisms at 20° C.

	Conc'n p.p.m.	Quaternary Ammonium Compounds				Hypochlorites	
		Roccal	R-2-L	Hyamine 1622	Emulsept	Dalglish	Klenzade X4
Gram-positive Species							
S. aureus	200	5	5	5	15	13	>20
M. candidus	200	5	5	5	7	>20	>20
B. panis — spores	1,000	3	1	1	15	>20	>20
— vegetative	100	5	5	1	5	1	5
Cheese starter	100	5	3	3	3	1	1
Flat sour organisms							
M 23	200	>40	..	..	..	>40	..
1503	200	>40	..	..	..	>40	..
1518	200	>40	..	..	..	>40	..
Gram-negative Species							
E. coli	200	15	10	>20	15	1	1
P. aeruginosa	200	5	5	8	8	1	5

ever, the number of spores present was so great that the 99.9 per cent end point had not been reached after 40 seconds' exposure. The sharp differences in resistance to quaternaries shown by Hucker to characterize the spore forms are not evident in the vegetative cells.

With the Gram-negative species, *E. coli* and *P. aeruginosa,* the hypochlorites are definitely superior, the advantage being greater than that in favor of the quaternaries against *S. aureus*. Incidentally, it is interesting to note that while other workers have reported *P. aeruginosa* as being much more resistant to quaternaries than is *E. coli,* our results with the glass slide technique have shown *Pseudomonas aeruginosa* to be the more easily killed. A possible explanation for this may be that the *P. aeruginosa* cultures contained a few highly resistant cells, so that, although 99.9 per cent destruction is accomplished in a relatively short time, yet the destruction of the last surviving cell may require a much stronger concentration or a longer exposure period than was necessary for *E. coli*. This point requires investigation.

Tests conducted against *S. aureus* at 45°C. indicated that there was some enhancement of germicidal activity of the quaternaries at the higher temperature, but the effect was slight compared with that noted for the hypochlorites. On the other hand, against *E. coli,* the quaternaries showed a more marked increase in activity at the higher temperature. Adjustment of the reaction to around pH 10 stimulated the activity of the quaternaries against *S. aureus* more than against *E. coli*; however, the effect was slight compared with that shown by the hypochlorites in solutions adjusted to pH 6.

Although it is not so evident from the data in Table 2, Emulsept was almost invariably less effective than were the other three quaternaries, while Klenzade X4 was similarly less effective than Dalglish.

It will be noted that the time required to destroy 99.9 per cent of the test organisms with the glass slide technique is a very small fraction of that required to effect complete sterilization with the F.D.A. technique or its modifications. Since in the glass slide technique the cells are present in a film of diluted skim milk, it seems unlikely that this difference is due entirely to a lesser amount of organic matter present than in the F.D.A. method. More probably it is due to (a) the smaller number of cells to be destroyed (varying between 8,000 and 160,000 per slide), and (b) the fact that while a very high percentage of the cells is usually destroyed within the first few seconds, an occasional cell survives for several minutes. The numbers present in the film in the glass slide technique are considerably higher than might be expected on the surfaces of washed food handling equipment and utensils,[5] while the amount of organic matter in the film of skim milk is also much greater. It would seem reasonable, therefore, to expect equally rapid destruction in actual plant operations.

In view of the recent evidence of strong bacteriostatic action displayed by some quaternary ammonium compounds in the F.D.A. technique,[1,2] considerable attention was given to the possibility that bacteriostasis might affect the results obtained with the glass slide technique. It was found that with the higher concentrations (used against spores of *B. panis*), quite marked inhibition of growth of *S. aureus* resulted, even where treated slides had received the customary dip in tap water to reduce the carry-over of residual germicide. With a concentration of 200 p.p.m. the zone of inhibition rarely exceeded the area of the slide; with weaker concentrations it was scarcely noticeable. This bacteriostatic effect was confined to the three Gram-positive species tested, even 1,000 p.p.m. having no effect upon the Gram-

negative species. The bacteriostatic effect was usually confined to the area above and adjacent to the treated slide, the test organisms growing freely on the remainder of the plate. While this effect was quite evident on a crowded plate, by the time the number of colonies approached the 99.9 per cent end point it was usually difficult to see much evidence of bacteriostasis. Consequently, it is believed that any error introduced by the bacteriostatic effect is slight compared with that in the F.D.A. method, and it is unlikely that such error has influenced the results to a significant degree. From a practical standpoint, this effect may be regarded as an advantage in that the residual film of a quaternary solution on a treated surface could be expected to continue its activity against any remaining organisms.

SUMMARY

To recapitulate, the glass slide appears to offer certain advantages in the evaluation of the efficiency of various types of sanitizing agents. Quaternary ammonium compounds, like chlorine compounds, show differences in their relative potency. In general, the former are more effective against the Gram-positive organisms, while the hypochlorites tested show an even greater advantage against the Gram-negative species tested. While the quaternary compounds show some responses to favorable adjustments in temperature and pH, these are much slighter than those shown by the hypochlorites.

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ADDENDUM

Since this paper was prepared, the following modifications have been adopted: (a) instead of receiving only a momentary dip, treated slides are agitated in tap water for 5 seconds before being plated; (b) control slides are shaken vigorously 25 times in 100 ml. sterile physiological saline solution in a 4 oz. screw-cap jar, the slide itself is plated out and 5 ml. of the saline plated separately. From these the total number of organisms originally present on the slide is calculated and this figure used in determining the end point of 99.9 per cent reduction.