Experimental Design and Statistical Analysis of an In-Use Test of Germicidal Detergents

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Abstract

Present methodology for the in-use testing of germicidal detergents is too time-consuming for routine use by a hospital environmentalist. A simplified experimental design and statistical analysis, amenable to routine use, is presented for the in-use testing of germicidal detergents against water alone. As an illustration of our methodology we evaluated two germicidal detergents versus water alone. Under our conditions of use, it was found that water alone was equally and significantly as effective ($p < 0.001$) as the two germicidal detergents in reducing microbial contamination of floors.

Introduction

A cause-effect relationship between microbial levels on environmental surfaces and the infection potential within a hospital has not been conclusively demonstrated. However, it is generally acknowledged that such contamination may play a role in hospital acquired infections. Hence, effective environmental control should be given a high priority.

A large number of germicidal detergents have been introduced in the last fifteen years. Tests for effectiveness of germicidal detergents fall into two large categories: in-use tests and laboratory tests (e.g., phenol coefficient, Rideal-Walker coefficient, use-dilution test). Laboratory tests cannot completely simulate the environment in and method by which a germicidal detergent is applied because conditions of actual use may differ greatly from those of a laboratory simulation. Factors which may differ include extraneous matter present on the surface, concentration of germicidal detergent, surface porosity, air temperature, relative humidity, contact time, water parameters (pH, hardness, temperature), and method of application. Therefore, it is necessary to test the effectiveness of a particular germicidal detergent under conditions which reflect actual use. The results of testing a product under in-use conditions cannot be generalized to other conditions of use. They are applicable only to the specific conditions under which a product was tested.

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A fundamental question confronting hospital environmentalists is whether or not a germicidal detergent is effective. In an effort to answer this question, several in-use test methods have been proposed (AYLIFFE et. al., 1967; GABLE 1966; KUIPERS 1968; LITSKY 1968; LITSKY, LITSKY 1968;OJAJARVI, MAHELA 1974; PALMER, YEOMAN 1972; VESLEY, MICHAELSEN 1964; VESLEY et. al., 1970). There is little comparability among these methods because different experimental designs and data analyses were used. Rigorous statistical techniques were not used to analyze data. In general the methods are quite time-consuming and therefore may not be amenable to routine use by a hospital environmentalist.

We present a simplified experimental design and rigorous statistical analysis of two experiments which compare the effectiveness of a phenolic germicidal detergent and a quaternary ammonium germicidal detergent to water alone. The index of effectiveness was the ability of a treatment to reduce the microbial density on a surface.

Materials and Methods

A. Experiment I

General Conditions of Use.

1. Treatments were prepared according to label instructions using 64^{o}C tap water for dilution (phenolic and quaternary both at ½ fl. oz. per gallon and water alone at 64^{o}C). Water at 64^{o}C is equivalent to using the hottest available tap water.

2. Non-sterile buckets were used to dilute the concentrated germicidal detergents.

3. There was no gross extraneous matter on the floor area studied, and no cleaning of floor tiles preceded application of treatments.

4. A new hand sponge was used to apply each treatment at 64^{o}C.

5. Walking on the test area was prohibited throughout the study.

No effort was made to insure bucket or sponge sterility, nor were the germicidal detergent solutions sterilized, as it is unlikely that sterile procedures would be used in routine hospital disinfection. However, the above conditions of use could be altered according to the practices of an individual facility. For example, the individual environmentalist may opt to use sterile procedures if in fact sterile procedures are used in routine disinfection.

The experimental design consisted of selecting four blocks, each consisting of three, nine-inch-square floor tiles. These tiles were located in a main hallway of an office-classroom building and were contaminated by student-faculty traffic. We would like to emphasize that any surface which is subject to the need for disinfection could be substituted. A treatment (phenolic, quaternary, water alone) was assigned at random to one of the three tiles in each block. Each tile was subdivided into nine, three-inch squares. Floor samples were taken randomly from four of the nine, three-inch squares within each tile before application of the treatments. Treatments were applied and the floor was allowed to dry. From the five remaining three inch squares within each tile, four floor samples were randomly obtained after the application of treatments. BBL RODAC pre-filled plates were used for floor sampling. These plates contain trypticase soy agar with lecithin and polysorbate 80 added for the neutralization of residual germicide. The RODAC plates were incubated 24 hours at 37°C and counted with standard Quebec colony counters. Total aerobic and facultative anaerobic organisms were recovered.

A 3-way analysis of variance was used to analyze the data. The three factors were treatment, block, and replication order (i.e. before or after treatment). An analysis of variance compensates for sample size, i.e. the larger the samples size, the smaller the F-ratio required for significance. Large sample sizes should be used only if an investigator is trying to demonstrate that small differences exist among treatments. Thus, the hospital environmentalist need not necessarily use the vast number of samples commonly recommended for surface sampling.

B. Experiment II

The treatment solutions (phenolic, quaternary, and water alone) were prepared using 24°C water. This is equivalent to using room temperature water. The experimental design consisted of selecting three blocks instead of four. The remainder of the experimental method and general conditions of use were as in Experiment I.

Results

TABLES 1 and 2 contain average colony counts per RODAC plate by block, treatment, and replication order (i.e. before vs. after treatment). TABLES 3 and 4 summarize the 3-way analysis of variance results.

TABLE 1 Average Colony Counts per RODAC Plate (4 in.^{2}) for Treatments Prepared with 64°C Water (Experiment I)

Block	Water Alone		Phenolic		Quaternary		Block
	Before	After	Before	After	Before	After	Mean
1	136.25	14.00	74.25	35.00	120.50	21.25	66.88
2	98.00	20.00	109.50	13.00	129.00	11.00	63.42
3	93.00	25.50	96.25	24.50	56.00	12.00	51.21
4	116.67	26.75	180.00	31.75	125.00	12.33	83.68
Treatment Mean	110.60	21.75	115.00	26.06	107.63	14.27	

TABLE 2 Average Colony Counts per RODAC Plate (4 in.^{2}) for Treatments Prepared with 24°C Water (Experiment II)

Block	Water Alone		Phenolic		Quaternary		Block
	Before	After	Before	After	Before	After	Mean
1	18.50	12.75	23.75	3.25	23.75	6.25	14.71
2	19.33	13.25	20.75	5.00	22.00	7.50	14.86
3	36.50	8.00	16.00	5.50	30.25	3.00	16.54
Treatment Mean	25.27	11.33	20.17	4.55	25.33	5.58	

TABLE 3 Analysis of Variance for Experiment I (64°C)

Source	Degrees of Freedom	Mean Square	F-Ratio	P-Value
Treatment	2	6.44	0.27	>0.25
Before/After	1	1917.43	81.63	<0.001
Block	3	36.34	1.54	>0.10
Interaction*	17	19.69	0.84	>0.25
Error	70	23.49	----	
TOTAL	93†	-----	----	

* Includes all two-factor and three-factor interactions.
† Two samples were discarded due to non-sterile agar.

TABLE 4

Analysis of Variance for Experiment II (24°C)

Source	Degrees of Freedom	Mean Square	F-Ratio	P-Value
Treatment	2	183.89	2.57	.05< P <.10
Before/After	1	4485.22	62.62	<0.001
Block	2	27.29	0.38	>0.25
Interaction*	12	121.70	1.70	.05< P <.10
Error	52	71.63	----	
Total	69†	-----	----	

* Includes all two-factor and three-factor interactions.
† Two samples were discarded due to non-sterile agar.

Experiment 1: At a significance level of 5%, there was no significant difference among the three treatments ($p > 0.25$). However, the reduction of the microbial densities on the floor was significant for all treatments ($p < 0.001$).

Experiment 2: At a significance level of 5%, there was no significant difference among the three treatments ($p > 0.05$). However, the reduction of the microbial densities on the floor was significant for all treatments ($p < 0.001$).

Discussion

The overall conclusions to be drawn from TABLES 3 and 4 are:

1. The three treatments do not significantly differ in their abilities to disinfect the floor surfaces tested. (If there was a statistically significant difference among the three treatments, multiple comparison statistical techniques could be used to identify the treatment(s) which differed).

2. The three treatments all significantly reduced the microbial density on the floor surfaces tested at either temperature.

It is important to stress that these conclusions apply only to the conditions of use in this study. We do not propose that our results be considered as a definitive comparison of the germicidal detergents used. Rather, we wish to emphasize the efficacy of the design and data analysis for surface sampling. Each hospital environmentalist must apply these techniques to his or her particular setting in order to judge the effectiveness of a particular germicidal detergent under their particular conditions of use.

The flexibility of the experimental design and analysis employed in this study is such that it can be adapted to various types of application techniques, the simultaneous evaluation of several germicidal detergents, various types of non-porous surfaces, and testing for residual germicidal effects. Its advantages arise from the simplicity of the design and data analysis.

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