

## Evaluation of disinfectant for microbial decontamination of the microbiology laboratory floor by an in-use test

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**ABSTRACT:**

A total of 80 samples of in-use disinfectant solutions from floor mop bucket were collected over four working weeks and bacterial contamination were measured by the *in-use* test technique. Phenolic compound represented by the brand name Crusade<sup>®</sup> was used at a concentration of 4 % (v/v), with Tween 80 in diluent of Salt Peptone Solution as the neutralizer. High average bacteria survival levels were recorded early, during three and six hours of disinfection of the floors throughout the four weeks study period, with an average bacterial count of log<sub>10</sub><sup>3</sup> cfu/ml. Later disinfection procedures at 9 and 24 hours resulted in the reduction in counts, with an average load of log<sub>10</sub><sup>2</sup> cfu/ml. Comparative mean counts (cfu/ml) per day of disinfection showed that the microbial load during disinfection was high at the beginning of each working week, usually on Mondays and Tuesdays with noticeable reductions through Wednesdays and then lower counts on Thursdays and Fridays of each week. The high levels detected each Monday during the four weeks may be attributed to bacteria build up over the two non working days of Saturdays and Sundays when no cleaning and disinfecting activities were undertaken. None of the samples taken met the satisfactory limit of less than 250 cfu/ml after 24 hours of incubation at 30°C for the *in-use* testing of working disinfectant.

**Keywords:**

Disinfectant, in-use test, microbial, decontamination, bacteria.

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## INTRODUCTION AND LITERATURE REVIEW

The significance of any product to the manufacturer with phenolic or hypochlorite content is based on demonstrated attainment of standardization. The choice of a brand name is thus chosen and followed by market promotion. For the laboratory practitioner involved in control of an infection however, the selection of the disinfection system, the product and the manufacturer's instructions regarding its use are paramount. Consequently the existing policy in the microbiology laboratory of the CSIR-Food Research Institute demands subjection to any new brand or batch of disinfectants for the assessment and thorough evaluation of its potency.

Emphasis has been focused on the performance of a wide variety of disinfectants based on their phenol co-efficient and other standardized tests (AOAC 1960, Kelsey and Sykes, 1969), than on their *in-use* capabilities (Kelsey and Maurer, 1966 and Maurer, 1978). The test previously used in most laboratories had been the Minimum Inhibitory Concentration (MIC), noted to be quite misleading despite the confidence placed in it (Maurer, 1978). The *in-use* testing of disinfectants in laboratories helps in monitoring their performance under local conditions and for a particular purpose.

Though disinfectants are not necessarily sterilizing agents, their use in testing laboratories where many microorganisms are encountered, can help to bring environmental contamination and pollution under control within the immediate surroundings. Although earlier investigations seemed to have originated from tests in hospitals (Maurer, 1978), the simple *in-use* test (Kelsey and Maurer, 1966; Maurer, 1978) has proved adequate for use in the microbiology laboratory.

Use of disinfectants has been described variously (Ostrander and Griffith, 1964; Public Health Laboratory Service Report, 1965; Ayliffe *et al.*, 1969; Prince and Ayliffe, 1972; Asiedu, 1992). The *in-use* dilutions are usually chosen from the manufacturers' recommendations or on the basis of the capacity test (Kelsey and Sykes, 1969). The *in-use* test is advisable to be performed for bacterial contamination (Kelsey and Maurer, 1966) on new disinfectants introduced to the laboratory; and should be evaluated at intervals for its potency and efficacy. These procedures should nevertheless be validated in an organized manner starting with laboratory assessment through to a controlled

evaluation over an extended period of time. This will produce an evidence-based data that will direct the selection to be made.

There are many types and formulations of disinfectants on sale in Ghana; the use of which are variously misused either due to unavailability of disinfection policy, or suitable instructions and training schedule for staff who work in such laboratories.

Some recommended disinfectants diluted in water and used in laboratories (Holton *et al.*, 1994; Griffiths *et al.*, 1998) in other countries include the following:

Sodium hypochlorite in concentrations of 1000 ppm (0.1%), 2,500 ppm (0.25%), 10,000 ppm (1%) or 20,000 ppm (2%) ([www.doh.gov.uk/cjd/tseguidance](http://www.doh.gov.uk/cjd/tseguidance)) and Chlorine releasing tablets and granules marketed as sodium dichloroisocyanurate (NaDCC). Hypochlorites are effective against vegetative bacteria, fungi and viruses, but with limited effectiveness against bacterial spores.

Tristel which is an aqueous solution of chlorine dioxide does not produce free chlorine and active against viruses, bacteria, fungi and their spores. At concentrations of 280 ppm, it is effective as rapid bactericide and sporocide than hypochlorite.

Alcohol is effective against many bacteria and fungi, with variable activity against viruses, but no activity against bacterial spores. The most effective concentration for disinfection is 70 – 80 % (v/v) solution of isopropanol or ethanol in water.

Aldehydes in the form of Formaldehyde and Glutaraldehyde. Formaldehyde gas is used in fumigation of microbiological safety cabinets and rooms. Glutaraldehyde causes dermatitis and respiratory problems. It is an asthmagen and therefore has a Maximum Exposure Limit (MEL) of only 0.05 ppm. It is therefore not recommended for use in an open laboratory but used as 2% Glutaraldehyde within safety cabinets that vent to the exterior and not recirculating so as to avoid recirculation within the confines of the laboratory (Holton *et al.*, 1994; Griffiths *et al.*, 1998). 1% Virkon, a multi-component peroxygen-based oxidizing agent is suitable for laboratories and effective against bacteria, fungi and viruses.

Testing disinfectants can be described as carrier test, suspension test, capacity test, practical test, field test or *in-use* test (Gardner and Peel, 1991; Rebrouck, 1998).

The carrier test (Robert Koch) has limitations due to difficulty in standardization of the



number of bacteria dried on a carrier and the survival of the bacteria on the carrier during drying. The test uses a carrier in the form of silk, catgut thread or a penicylinder (a little stick) contaminated by submersion in liquid culture of a test organism. The contact is then dried, disinfected for a specified time, then cultured in nutrient broth. No growth indicates disinfectant activity while growth indicates inactivity of the disinfectant. An active concentration-time relationship is derived from multiplying the number of test concentrations of the disinfectant and the contact times.

AOAC use-dilution carrier-based test (1990) uses sterile stainless steel cylinders immersed in suspension of test organism of either *Salmonella choleraesuis*, *Staphylococcus aureus* or *Pseudomonas aeruginosa*. After the cylinders are drained on filter paper and dried at 37°C for 40 minutes, they are exposed for 10 minutes in the *in-use* dilution of the disinfectant. The use-dilution test is used to confirm the efficiency of disinfectant dilution derived from phenol coefficient test. The phenol coefficient of a disinfectant is calculated by dividing the dilution of test disinfectant by the dilution of phenol that disinfects under predetermined conditions.

Suspension tests require a sample of the bacterial culture uniformly suspended in the disinfectant solution which after exposure is verified by subculture to ascertain whether the inoculum was killed or not.

The best known capacity test (Kelsey and Sykes, 1969) is designed to determine concentrations of disinfectant that will be effective in clean and dirty conditions, using test organisms such as *S. aureus*, *P. aeruginosa*, *P. vulgaris* and *E. coli*.

An *in-use* test (Maurer, 1978) used in hospitals and laboratories to detect disinfectant contamination employs 1.0 ml sample of the disinfectant in 9.0 ml diluent which also contains an activator. Then 0.02 ml (10 drops) of the diluted sample is placed on each of duplicate nutrient agar plates. One is incubated at 37°C for 3 days while the other at room temperature for 7 days. Contamination is indicated by 5 or more colonies on either plate.

In this study, the effectiveness of a disinfectant used in the microbiology laboratory for bacterial decontamination was evaluated by an *in-use* test (Kelsey and Maurer, 1966; Maurer, 1978).

## MATERIALS AND METHODS

### Study areas

The study areas were Inoculation Rooms one and two which are located in the inner periphery of the Microbiology laboratory of the Food Research Institute. It is enclosed within the main laboratory, and separated by a wooden door from the other rooms. Inoculation room one is used for general analysis related to non-pathogenic microorganisms while room two is reserved for work on pathogenic microorganisms. The dimension of the total working area of each room measures 3.0 m in width by 4.5 m in length.

### Materials

Eighty samples of *in-use* disinfectant solutions were taken over a period of four weeks at intervals of 3, 6, 9 and 24 hours.

### Disinfectant

The phenolic synthetic detergent-disinfectants used in the study were 4-chloro-3,5-dimethylphenol or Parachlorometaxylenol (PCMX) and also 4-dichloro-3,5-dimethylphenol or Dichlorometaxylenol (DCMX). The detergents indicated a Rideal Walker Test Value of 4.2 as per information supplied by the manufacturer located in Accra, Ghana. Four percent (v/v) solutions recommended by the manufacturer for floor mopping were constituted with sterile distilled water for the *in-use* test.

### Floor-mop bucket, floor-mop head and mopping procedure

The plastic buckets were sterilized before use. They were autoclaved prior to the opening being covered with newsprint that is held tightly with cellophane tape. Two floor-mop heads which are used alternately were wrapped in grease paper and also autoclaved. Each bucket contained 5.0 litres of freshly prepared *in-use* solution. The wooden handle of the mop heads were swabbed severally with cotton wool soaked in 70 % alcohol. The usual practice in the laboratory is to mop the floor with detergent solution before commencement of analysis. The practice was followed in the study by dipping the mop head in the *in-use* disinfectant solution in the bucket and then ran severally over the terrazzo floor surface of the inoculation rooms; before rinsing in the solution in the bucket and squeezing the mop head to remove excess fluid. The procedure was repeated until the whole floor area was disinfected.

### Media

Media prepared were Salt Peptone Solution

(SPS) composed of Sodium Chloride (8.5g), Peptone (1.0g), Distilled water (1000 ml), pH ( $7.2 \pm 0.2$ ), Tween 80 [Polyoxyethylene (20) Sorbitan Monooleate] as 3% neutralizer/inactivator; and also Nutrient Agar composed of Lab lemco powder (1.0 g), Yeast Extract (2.0 g), Peptone (5.0 g), Sodium Chloride (5.0 g), Agar (15.0 g), Distilled water (1000 ml), maintained at pH  $7.4 \pm 0.2$ .

### Equipment

Equipment used are Incubator (Memmert, GMBH, Germany, model ICP 600 set at 30°C), Autoclave (Prioclave Ltd., model PS/LAC/EN 150), Hot air oven (Elektroheliol, model 28562) and Micropipette (Finnpipette of capacity 20 - 200µl and 100 - 1000µl, Labsystem, model 4,500). Other equipment were buckets, mops, Petri dishes and test tubes.

### Methodology

The method of Kelsey and Maurer (1966) was used.

### Test Requirements

Proximity of the test sites (study area) and the Nutrient Agar plates allowed for the samples to be analyzed within one hour, as a requirement of the *in-use* test method.

### Sampling

By means of a micropipette, 1.0 ml of the *in-use* disinfectant solution was transferred into a test tube of 9.0 ml sterile diluent (Salt Peptone Solution) with 3 % Tween 80 (Polyoxyethylene Sorbitan Monooleate) as neutralizer. This was thoroughly mixed by Vortex to obtain a homogeneous solution.

### Inoculation and incubation of plates

A calibrated micropipette, delivering drops of 20 µl was used to withdraw and drop the disinfectant and diluent solution at 10 separate points equidistantly on the surface of Nutrient Agar plates that had been dried at 55°C for 45 minutes. The plates were allowed to stand for 15 minutes for the drops to be absorbed before incubating them inverted. The inoculated Nutrient Agar plates were incubated at 30 °C for 72 hours in an incubator (Memmert GMBH model ICP 600, Germany).

### Calculation of Colony Forming Units

The total count was recorded as cfu/ml. This was calculated using the formula given below:

$$\text{cfu/ml} = \frac{\text{Total Count of 10 drops}}{10} \times 50 \times \text{Dilution factor}$$

## RESULTS AND DISCUSSION

### Average bacteria survival in a working week in the laboratory

The results in cfu/ml (Miles and Misra) of *in*

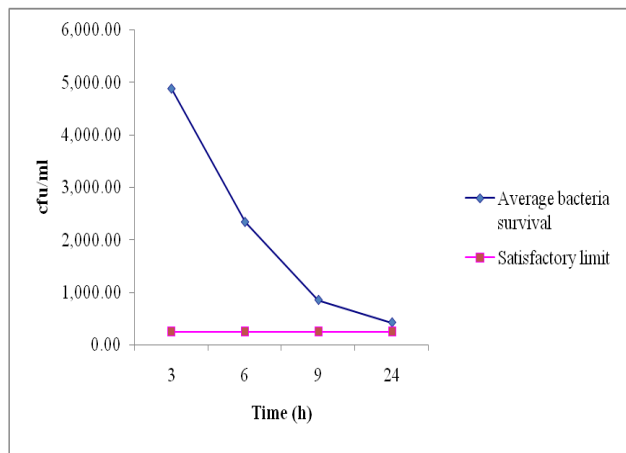
*-use* test disinfectant solutions on Nutrient Agar are as shown in **Table 1**. High average bacteria survival levels were recorded at the early hours of disinfection of the floors at three and six hours throughout the study period (Table 1), with an average bacterial count of  $\log_{10}^3$  cfu/ml. Reduction in counts was observed for 9 and 24 hours for the four weeks, with an average load of  $\log_{10}^2$  cfu/ml of disinfectant solution (Table 1).

Comparative mean counts (cfu/ml) per day of disinfection are as shown in **Table 2**. The microbial load during disinfection was high at the beginning of each working week, usually on Mondays and Tuesdays with noticeable reductions through Wednesdays and then lower counts recorded on Thursdays and Fridays of each week. The high levels detected on Mondays in particular could be attributed to bacteria build up over the two non-working days of Saturdays and Sundays when no cleaning and disinfecting activities were undertaken in the laboratory.

**Figure 1** shows presentation of average bacteria survival in *in-use* disinfectant solution (from mop bucket) at 3, 6, 9 and 24 hour intervals. 24 hours for each week. **Figures 2** shows comparative average bacteria survival in *in-use* disinfectant solution (from mop bucket) sampled between 3.

**Table 3** shows the average percentage reduction in bacterial survival at 3, 6, 9 and 24 hours in relation to the first day of the week when disinfection begun. At 3 hours, between Monday and Friday 50.27 % reduction in bacteria survival was recorded; with reduction on Tuesday, Wednesday and Thursday recording 13.51, 38.92 and 46.49 % respectively. At 6 hours, percentage

**Fig. 1. Average bacteria survival in *in-use* disinfectant solution (from mop bucket) at 3, 6, 9 and 24 hour intervals**





**Table 1: Bacteria Count on Nutrient Agar of *in-use* test disinfectant samples in inoculation rooms 1 and 2 at intervals of 3, 6, 9 and 24 hours**

Working day	Inteval of sapling (h)	Counts on NA Plates	No of drops of disinfectant per plate	Average Count on NA/drop	Average Count per drop x 50 (Miles & Misra)	Dilution factor	Colony Forming Units (cfu/ml)
<b>Week 1</b> Monday	3	135	10	13.5	675	10 <sup>1</sup>	6.8 x 10 <sup>3</sup>
	6	67	10	6.7	335	10 <sup>1</sup>	3.4 x 10 <sup>3</sup>
	9	30	10	3.0	150	10 <sup>1</sup>	1.5 x 10 <sup>3</sup>
	24	11	10	1.1	55	10 <sup>1</sup>	5.0 x 10 <sup>2</sup>
Tuesday	3	125	10	12.5	625	10 <sup>1</sup>	6.3 x 10 <sup>3</sup>
	6	59	10	5.9	295	10 <sup>1</sup>	3.0 x 10 <sup>3</sup>
	9	25	10	2.5	125	10 <sup>1</sup>	1.3 x 10 <sup>3</sup>
	24	10	10	1.0	50	10 <sup>1</sup>	5.0 x 10 <sup>2</sup>
Wednesday	3	80	10	8.0	400	10 <sup>1</sup>	4.0 x 10 <sup>3</sup>
	6	39	10	3.9	195	10 <sup>1</sup>	2.0 x 10 <sup>3</sup>
	9	18	10	1.9	90	10 <sup>1</sup>	9.0 x 10 <sup>2</sup>
	24	7	10	0.7	35	10 <sup>1</sup>	3.5 x 10 <sup>2</sup>
Thursday	3	77	10	7.7	385	10 <sup>1</sup>	3.9 x 10 <sup>3</sup>
	6	41	10	4.1	205	10 <sup>1</sup>	2.1 x 10 <sup>3</sup>
	9	17	10	1.7	85	10 <sup>1</sup>	8.5 x 10 <sup>2</sup>
	24	8	10	0.8	40	10 <sup>1</sup>	4.0 x 10 <sup>2</sup>
Friday	3	70	10	7.9	350	10 <sup>1</sup>	3.5 x 10 <sup>3</sup>
	6	33	10	3.3	165	10 <sup>1</sup>	1.7 x 10 <sup>3</sup>
	9	18	10	1.8	90	10 <sup>1</sup>	9.0 x 10 <sup>2</sup>
	24	8	10	0.8	40	10 <sup>1</sup>	4.0 x 10 <sup>2</sup>
<b>Week 2</b> Monday	3	144	10	14.4	720	10 <sup>1</sup>	7.2 x 10 <sup>3</sup>
	6	59	10	5.9	295	10 <sup>1</sup>	3.0 x 10 <sup>3</sup>
	9	28	10	2.8	140	10 <sup>1</sup>	1.4 x 10 <sup>3</sup>
	24	12	10	1.2	60	10 <sup>1</sup>	6.0 x 10 <sup>2</sup>
Tuesday	3	1230	10	12.3	615	10 <sup>1</sup>	6.2 x 10 <sup>3</sup>
	6	52	10	5.2	260	10 <sup>1</sup>	2.6 x 10 <sup>3</sup>
	9	22	10	2.2	110	10 <sup>1</sup>	1.1 x 10 <sup>3</sup>
	24	10	10	1.0	50	10 <sup>1</sup>	5.0 x 10 <sup>2</sup>
Wednesday	3	69	10	6.9	345	10 <sup>1</sup>	3.5 x 10 <sup>3</sup>
	6	36	10	3.6	180	10 <sup>1</sup>	1.8 x 10 <sup>3</sup>
	9	15	10	1.5	75	10 <sup>1</sup>	7.5 x 10 <sup>2</sup>
	24	7	10	0.7	35	10 <sup>1</sup>	3.5 x 10 <sup>2</sup>
Thursday	3	71	10	7.1	355	10 <sup>1</sup>	3.6 x 10 <sup>3</sup>
	6	33	10	3.3	165	10 <sup>1</sup>	1.7 x 10 <sup>3</sup>
	9	15	10	1.5	75	10 <sup>1</sup>	7.5 x 10 <sup>2</sup>
	24	6	10	0.6	30	10 <sup>1</sup>	3.0 x 10 <sup>2</sup>
Friday	3	64	10	6.4	320	10 <sup>1</sup>	3.2 x 10 <sup>3</sup>
	6	29	10	2.9	145	10 <sup>1</sup>	1.5 x 10 <sup>3</sup>
	9	13	10	1.3	65	10 <sup>1</sup>	6.5 x 10 <sup>2</sup>
	24	71	10	7.1	35	10 <sup>1</sup>	3.5 x 10 <sup>2</sup>



<b>Week 3</b> Monday	3	147	10	14.7	735	10 <sup>1</sup>	7.5 x 10 <sup>3</sup>
	6	70	10	7.0	350	10 <sup>1</sup>	3.5 x 10 <sup>3</sup>
	9	16	10	1.6	80	10 <sup>1</sup>	8.0 x 10 <sup>2</sup>
	24	12	10	1.2	60	10 <sup>1</sup>	6.0 x 10 <sup>2</sup>
Tuesday	3	127	10	12.7	635	10 <sup>1</sup>	6.4 x 10 <sup>3</sup>
	6	60	10	6.0	300	10 <sup>1</sup>	3.0 x 10 <sup>3</sup>
	9	14	10	1.4	70	10 <sup>1</sup>	7.0 x 10 <sup>2</sup>
	24	10	10	1.0	50	10 <sup>1</sup>	5.0 x 10 <sup>2</sup>
Wednesday	3	107	10	10.7	535	10 <sup>1</sup>	5.4 x 10 <sup>3</sup>
	6	55	10	5.5	275	10 <sup>1</sup>	2.8 x 10 <sup>3</sup>
	9	11	10	1.1	55	10 <sup>1</sup>	5.5 x 10 <sup>2</sup>
	24	6	10	0.6	30	10 <sup>1</sup>	3.0 x 10 <sup>2</sup>
Thursday	3	80	10	8.0	400	10 <sup>1</sup>	4.0 x 10 <sup>3</sup>
	6	38	10	3.8	190	10 <sup>1</sup>	1.9 x 10 <sup>3</sup>
	9	15	10	1.5	75	10 <sup>1</sup>	7.5 x 10 <sup>2</sup>
	24	6	10	0.6	30	10 <sup>1</sup>	3.0 x 10 <sup>2</sup>
Friday	3	76	10	7.6	380	10 <sup>1</sup>	3.8 x 10 <sup>3</sup>
	6	31	10	3.1	155	10 <sup>1</sup>	1.6 x 10 <sup>3</sup>
	9	13	10	1.3	65	10 <sup>1</sup>	6.5 x 10 <sup>2</sup>
	24	7	10	0.7	35	10 <sup>1</sup>	3.5 x 10 <sup>2</sup>
<b>Week 4</b> Monday	3	129	10	12.9	645	10 <sup>1</sup>	6.5 x 10 <sup>3</sup>
	6	67	10	6.7	335	10 <sup>1</sup>	3.4 x 10 <sup>3</sup>
	9	14	10	1.4	70	10 <sup>1</sup>	7.0 x 10 <sup>2</sup>
	24	11	10	1.1	55	10 <sup>1</sup>	5.5 x 10 <sup>2</sup>
Tuesday	3	105	10	10.5	525	10 <sup>1</sup>	5.3 x 10 <sup>3</sup>
	6	64	10	6.4	320	10 <sup>1</sup>	3.2 x 10 <sup>3</sup>
	9	12	10	1.2	60	10 <sup>1</sup>	6.0 x 10 <sup>2</sup>
	24	9	10	0.9	45	10 <sup>1</sup>	4.5 x 10 <sup>2</sup>
Wednesday	3	83	10	8.3	415	10 <sup>1</sup>	4.2 x 10 <sup>3</sup>
	6	37	10	3.7	185	10 <sup>1</sup>	1.9 x 10 <sup>3</sup>
	9	16	10	1.6	80	10 <sup>1</sup>	8.0 x 10 <sup>2</sup>
	24	7	10	0.7	35	10 <sup>1</sup>	3.5 x 10 <sup>2</sup>
Thursday	3	69	10	6.9	345	10 <sup>1</sup>	3.5 x 10 <sup>3</sup>
	6	28	10	2.8	170	10 <sup>1</sup>	1.7 x 10 <sup>3</sup>
	9	12	10	1.2	70	10 <sup>1</sup>	7.0 x 10 <sup>2</sup>
	24	6	10	0.6	35	10 <sup>1</sup>	3.5 x 10 <sup>2</sup>
Friday	3	66	10	6.6	330	10 <sup>1</sup>	3.3 x 10 <sup>3</sup>
	6	28	10	2.8	140	10 <sup>1</sup>	1.4 x 10 <sup>3</sup>
	9	12	10	1.2	60	10 <sup>1</sup>	6.0 x 10 <sup>2</sup>
	24	6	10	0.6	30	10 <sup>1</sup>	3.0 x 10 <sup>2</sup>





Table 2. Comparative mean counts (cfu/ml) per day of disinfection

Day	Week	Microbial load at time of disinfection			
		HRS	6HRS	9HRS	4HRS
Monday	Wk 1	6,750	3,350	1,500	550
Monday	Wk 2	7,200	2,960	1,400	600
Monday	Wk 3	7,350	3,500	800	600
Monday	Wk 4	6,450	3,350	700	550
	<b>TOTAL</b>	<b>27,750</b>	<b>13,160</b>	<b>4,400</b>	<b>2300</b>
	<b>MEAN</b>	<b>6,937.50</b>	<b>3,287.50</b>	<b>1,100</b>	<b>575</b>
Tuesday	Wk 1	6,250	2,950	1,250	500
Tuesday	Wk 2	6,150	2,600	1,100	500
Tuesday	Wk 3	6,350	3,000	700	500
Tuesday	Wk 4	5,250	3,200	600	450
	<b>TOTAL</b>	<b>24,000</b>	<b>11,750</b>	<b>3,650</b>	<b>1950</b>
	<b>MEAN</b>	<b>6,000</b>	<b>2,937.50</b>	<b>912.5</b>	<b>487.5</b>
Wednesday	Wk 1	4,000	1,950	900	350
Wednesday	Wk 2	3,450	1,800	750	350
Wednesday	Wk 3	5,350	2,750	550	300
Wednesday	Wk 4	4,150	1,850	800	350
	<b>TOTAL</b>	<b>16,950</b>	<b>8,350</b>	<b>3000</b>	<b>1350</b>
	<b>MEAN</b>	<b>4,237.50</b>	<b>2,087.50</b>	<b>750</b>	<b>337.5</b>
Thursday	Wk 1	3,850	2,050	850	400
Thursday	Wk 2	3,550	1,650	750	300
Thursday	Wk 3	4,000	1,900	750	300
Thursday	Wk 4	3,450	1,700	700	350
	<b>TOTAL</b>	<b>14,850</b>	<b>7,300</b>	<b>3050</b>	<b>1350</b>
	<b>MEAN</b>	<b>3,712.50</b>	<b>1,825</b>	<b>762.5</b>	<b>337.5</b>
Friday	Wk 1	3,500	1,650	900	400
Friday	Wk 2	3,200	1,450	650	350
Friday	Wk 3	3,800	1,550	650	350
Friday	Wk 4	3,300	1,400	600	300
	<b>TOTAL</b>	<b>13,800</b>	<b>6,050</b>	<b>2800</b>	<b>1400</b>
	<b>MEAN</b>	<b>3,450</b>	<b>1,512.50</b>	<b>700</b>	<b>350</b>
<b>Mean HOURS</b>		<b>4,867.50</b>	<b>2,329.90</b>	<b>845</b>	<b>417.5</b>

bacteria survival recorded between Monday and Friday were 10.65, 36.50, 44.49 and 46.00 % respectively. At 9 h, bacterial survival was 17.05, 31.82, 30.68, 36.37 % while 15.22, 41.30, 41.30, 39.13 % were recorded for 24 hours. Generally, there was a decreasing trend in bacteria from the beginning to the end of the week for all the time investigated.

**Table 4** shows the average percentage reduction in bacterial survival between 3 – 6, 6 – 9

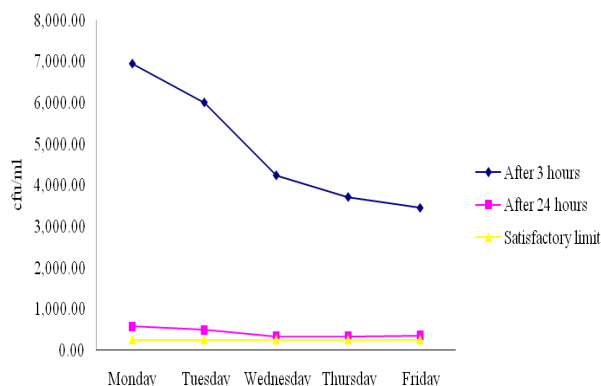
and 9 – 24 hours of a day. It was observed that bacteria reduction between three and six hours of disinfection from Monday to Friday was 52.61, 51.04, 50.74, 50.84 and 56.16 % respectively. Between 6 and 9 hours recorded 66.54, 68.94, 64.07, 58.22, 53.72 % while between 9 and 24 hours, 47.73, 46.58, 55.00, 55.74 and 50 % reduction from Monday to Friday were observed.

#### Satisfactory Limits

From the results obtained, none of the



**Fig. 2. Comparative average bacteria survival in in-use disinfectant solution (from mop bucket) between 3 and 24 hours for each week**



samples irrespective of the interval met the satisfactory limit of less than 250cfu/ml after 24 hours as recommended by Kelsey and Maurer (1966) and Collins and Lyne (1984).

In this study, the choice of exposure time is essential to the effectiveness of the disinfectant. Kelsey and Maurer (1966) employed an *in-use* test involving sampling at the end of the day. In this study 9 hours was used as the length of a day’s activities in the laboratory. However, Collins and Lyne (1984) suggested that irrespective of local policy (whether emptied at the end of the day or the following morning), *in-use* disinfectant solutions should not be allowed to stand for more than 24 hours hence the need for the investigation to cover the maximum allowable time of 24 hours in this study.

The initial bacteria loads picked from the floor through cleaning and mopping varied between the various days. On the first sampling after three hours, the number of bacteria surviving in the disinfectant solutions of the floor mop bucket was extremely high. After 9 hours, the surviving

population levels were still unacceptably high. Even after 24 hours none of the results recorded for any of the days met the satisfactory standards recommended by Kelsey and Maurer (1966) of less than 250 cfu/ml. It was therefore observed that the disinfectant per the manufacturer’s recommended concentration was unable to disinfect the floor satisfactorily. It was also difficult to quantify the bacterial load introduced from the floor through the mopping into the disinfectant solution. It was therefore essential that the longest possible exposure time of 24 hours be chosen so as to reduce the hazard of higher percentage of surviving micro-organisms occurring. It would therefore be recommended that from the findings, effective disinfection of the microbiology floor would have to be carried out with prolonged time of exposure to the disinfectant for any meaningful gains to be achieved; that is if continued use of the same brand of disinfectant would be carried out. Christensen *et al.*, (1982) used a 2 % (v/v) concentration of a phenolic disinfectant, Bacillotox ® compound in *in-use* tests with remarkable results which met the satisfactory standard of less than 250 cfu/ml after 24 hours exposure (Kelsey and Maurer, 1966; Collins and Lyne, 1984). Depending on the results of this study, it would be desirable to examine the efficiency of other locally available disinfectants so as to make a choice based on efficacy and cost. Another alternative would be to engage the services of commercial cleaning professionals after evaluation of their performance based on satisfactory *in-use* test results.

For a disinfectant to be effective, adequate contact with the surface with avoidance of air pockets should be ensured. Where objects are concerned, full immersion should be done; and deposits of organic matter must be removed prior to disinfection. Factors that affect the effectiveness of

**Table 3. Average percentage reduction in bacterial survival between days of the week in in-use disinfectant solution from 3, 6, 9 and 24 hours**

Day	Percentage Reduction in Bacteria Survival (%)			
	3h	6h	9h	24h
Monday	0	0	0	0
Tuesday	13.51	10.65	17.05	15.22
Wednesday	38.92	36.50	31.82	41.30
Thursday	46.49	44.49	30.68	41.30
Friday	50.27	46.00	36.37	39.13

**Table 4. Average percentage reduction in bacterial survival between hours of the day**

Day	Percentage Reduction in Bacteria Survival (%)		
	3 - 6 h	6 – 9 h	9 – 24 h
Monday	52.61	66.54	47.73
Tuesday	51.04	68.94	46.58
Wednesday	50.74	64.07	55.00
Thursday	50.84	58.22	55.74
Friday	56.16	53.72	50.00





disinfectants are organic matter, time, light and temperature of exposure. The contact time of disinfection should be adequate for good performance of the disinfectant. This time varies in respect of the disinfectant type, the microbial load and presence of factors that inactivate or interfere (e.g. excessive organic material and/or chemicals). Although disinfectants do not necessarily kill all biological agents and do not usually destroy bacterial spores, it would be nevertheless more effective to always use freshly prepared *in-use* dilutions to disinfect laboratory floors and surfaces since stored diluted disinfectant solutions may lose potency.

### CONCLUSION AND RECOMMENDATIONS

The study showed that the recommended concentration of 4 % (v/v) by the manufacturer was not adequate enough to reduce the microbial load even after 24 hours of disinfection. In this regard, it is recommended that

- Disinfectants available locally should be tested so that choices could be made and adopted for all laboratories dependent on their efficacy in the *in-use* test results.
- Since the services of professional cleaners are now readily available in Ghana, it is recommended that the cleaning agents they use should be evaluated as well as their performance within four weeks in order to ascertain the efficacy.
- It is recommended that the continued use of this particular disinfectant should be based on review of increasing the concentration for effectiveness in its use.
- Only freshly prepared *in-use* dilutions should be used to disinfect laboratory floors and surfaces since stored diluted disinfectant solutions may lose their potency.

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