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IN-USE TESTS OF DISINFECTANTS

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BY

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REG. NO

IN PARTIAL FULFILMENT FOR THE AWARD OF
HIGHER DIPLOMA IN MICROBIOLOGICAL TECHNIQUES

FOOD RESEARCH INSTITUTE
(COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH)

DECLARATION

I hereby declare that this project was done by me in February 1993 at the Food Research Institute (Council for Scientific and Industrial Research) of Ghana under the supervision of Prof. G.C. Clerk, Department of Botany, University of Ghana.

Signature:.....
STUDENT

Signature:.....
SUPERVISOR

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The Ghana Standards Board Library
The Balme (Univeristy of Ghana) Library
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The British Council Library

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C O N T E N T S

FIGURES

PLATES

ABSTRACT

The method described by Kelsey and Maurer (1966) and Maurer (1978) was employed in the 'in-use' testing of working disinfectants from four selected sites in a Food Microbiology Laboratory. The sites were:

1. Discard Jar
2. Discard Culture Spreader Jar
3. Discard Pipette Jar
4. Floor-Mop Bucket.

Phenolic compound represented by brand name, Crusade$_{(R)}$ was used at 10% concentration. The neutralizer was Tween 80 in quarter strength Ringer's Solution. In all 80 samples of the disinfectant was collected over one week, from 15th to 19th February, 1993. Results of Nine samples collected at the end of the day (9 hours in my study) were within the Kelsey & Maurer satisfactory count limit.

Fifteen more samples which were collected just before the in-use solutions were to be discarded - 24 hours - also fell within the limit.

The study showed that it is possible to conduct a simple in-use testing of disinfectants in any laboratory where tests sites similar to those in the study are being used.

A higher concentration of 10% (V/V) of the disinfectant was used instead of the 4% (V/V) recommended by the manufacturer which proved completely ineffective in preliminary studies. It has been recommended that the continued use of this particular disinfectant should be reviewed.

Aerobic sporing and non-sporing Bacillus Species, Lactobacillus Species, and Staphylococcus albus, all Gram positive organisms, were identified. In addition, the fungi, Aspergillus niger and species of Penicillium were isolated on a Potato Dextrose Agar plate on one occasion.

CHAPTER ONE

INTRODUCTION

Comparatively, more emphasis seemed to have been focussed on the performance of various disinfectants based on their phenol co-efficient and other standardized tests (AOAC 1960, Kelsey & Sykes 1969), than on their 'in-use' test results suggested by Kelsey & Maurer (1966) and Maurer (1978).

The significance of a product whether phenolic or Hypochlorite to manufacturer is when it has been demonstrated to have passed the standardized tests. The subsequent step is the choice of a brand name followed by market promotion. For the laboratory practitioner, the manufacturer's instructions regarding its use is deemed adequate. However, existing policies in some laboratories demand the subjection of any new brand or batch to be introduced, to some of the tests mentioned earlier. The test often used in most laboratories is the Minimum Inhibitory Concentration (MIC). This particular test (MIC) has been noted to be quite misleading despite the confidence placed on it (Maurer, 1978).

The in-use testing of disinfectants being used in the laboratories is quite valuable as it will help monitor their performance under local conditions and for particular purposes.

In a food microbiology laboratory, one would be tempted to relegate to the background the presence of pathogens but the situation can be real. For example, Salmonella species have very often been isolated from poultry products (Roberts, 1972).

Though disinfectants are not necessarily sterilizing agents, their use in laboratories where many organisms are handled can help to bring contamination and pollution under control.

The simple in-use test suggested by Kelsey & Maurer (1966) and Maurer (1978) should be possible, if not obligatory, in every laboratory although most reports of earlier works seemed to have originated from tests in hospitals (Maurer, 1978; Christensen, et. al. 1982).

The objectives of this study are:

1. To determine the effectiveness of the 'in-use' disinfectant in the mircobiology laboratory of the Food Research Institute.

2. To determine the level (counts) of microorganisms surviving at different intervals after introduction into the disinfectant.

3. To Isolate and identify the organisms

PLATE 1: PHOTOGRAPH SHOWING THE LAYOUT OF THE MICROBIOLOGY LABORATORY (X 1/22)

CHAPTER TWO

LITERATURE REVIEW

The terms 'Sterilization' and 'Disinfection' are very often distinguished from each other by authors dealing with the subject. Whereas sterilization is defined as destruction of all forms of life (Hayes, 1985), disinfection means the destruction of vegetative cells which might cause disease (Collins & Lyne, 1984). Disinfection thus implies partial removal of contaminants, but endospores of bacteria often survive the treatment.

The study of the performance of various disinfectants (Phenols, Quaternary Ammonium Compounds, Aldehydes, Hypochlorites, Alcohols, etc.) have led to the establishment of selective effectiveness against categorized organisms (WHO, 1983). Maurer (1969) showed that bacteria do not only survive but multiply in some disinfectant solutions. Susceptibility to phenolic disinfectant varies greatly. Indeed, some microorganisms use phenol as food (Frobisher 1968).

Besides effectiveness, toxicity indices are established for each brand of disinfectant based on their effect on tissue, skin, eyes and the respiratory tract. Precautions in the use of disinfectants has been stressed (Collins & Lyne, 1984). Safety kits - spectacles goggles or visor and disposable gloves are to be worn when handling strong disinfectant solutions.

The Rideal-Walker test and subsequent modifications (British standard 541, 1934) and further amendments is still widely used in the routine quality control check of batches of phenolics (phenol co-efficient).

The method of Chick-Martin seemed more efficient than the Rideal-Walker test. The Chick-Martin method has however, also undergone some modifications (British Standard, 808, 1939). The Chick-Martin method corrected the weakness in the Rideal-Walker test by principally incorporating an organic material in the test mixture. It is well established that the presence of organic materials interfere with the action of disinfectants. One other defect of the Rideal- Walker test is that the results are specific for the test organisms used, ie. Salmonella typhi (Croshaw, 1981).

Other tests, AOAC (1960) and Kelsey-Sykes (1969) are currently employed in the evaluation of both phenolic and non-phenolic disinfectants.

Despite the critical review by Cowen (1978) for its unsuitability to many types of disinfectants the modified Kelsey-Sykes method by Kelsey and Maurer (1974) has been adopted by official bodies.

In-Use Application

Prepared disinfectant solutions for use in the laboratory are not stored over 24 hours (Collins & Lyne, 1984). Beyond this limit, the solution declines in potency and many organisms survive.

Contaminated disinfectant solutions have been associated with the outbreak of Pseudomonas aeruginosa infection (Jellard & Churcher, Gillian, 1967). A phenolic disinfectant/detergent in-use solution containing between one million and ten million Pseudomonas per ml. in routine in-use test has been reported (Givan, Black and williams, 1971). Hypochlorites, phenols, glutaraldehyde, formalin, chlorohexidine, chloroxylenol and quaternary ammonium compunds have all been incriminated in the outbreak of infection (Maurer, 1978).

Christensen et. al, (1982) compared the Kelsey - Maurer test with

two other test methods: (a) Modified Kelsey - Maurer in-use test by membrane filtration, and (b) Challenge test by means of standardised preparations of bacteria or fungi, in tests carried out at two different laboratories. It was concluded that modification of Kelsey & Maurer by the introduction of membrane filtration eliminated the need for neutralizing agents in the media. Consequently, interference with antimicrobial activity of the disinfectant is prevented.

It seems, therefore, that results of the Kelsey - Maurer test may not always reflect the efficiency of the disinfectant since there are other causes of variation in results. For example, very few or even no microorganisms may have been present on the articles immersed in the solutions. The exposure time could also have been far longer than the minimum exposure time recommended for the particular disinfectant. In these instances the absence of organisms on the Nutrient Agar plates does not necessarily demonstrate sufficient antimicrobial efficiency of the disinfectant. A standardized challenge test is therefore useful in verifying results obtained by the Kelsey - Maurer test.

Good planning and implementation of disinfection policy (Prince, Jean and Ayliffe, 1972) can reduce to minimum the outbreak of infections and to cut cost (Maurer, 1978).

CHAPTER THREE

MATERIALS AND METHODS

1. LOCATION

The investigation and isolations were carrived out at the Food Research Institute of Ghana with the identifications at Noguchi Memorial Institute for Medical Research while the planning of the project took place at the Botany Department of the University of Ghana, Legon.

All the three institutions are sited in Accra the capital city of Ghana. Though Accra is found along the Atlantic Coast, the vegetation of the Greater Accra Region is mostly of the Savannah type. The mean rainfall is between 700-900mm. The highest mean temperature is about 30^{0}C between the months of March and April and the lowest mean of about 25.8°C in August. The average monthly relative humidity ranges between 60 and 72% R.H.

2. STUDY AREAS

(a) Food Research Institute

The Food Research Institute is located behind the Ghana Police Headquarters, 5th Rangoon Close on Rangoon Avenue, off Ring-Road Central.

The Institute administrative offices and majority of the laboratories are housed in single storey buildings. The Microbiology Department where the investigation was conducted is about 50 metres away from the main buildings. It is a two-storey building with two

Microbiology Laboratories (Lactic acid & General). The lactic acid laboratory was selected as one of the sites - Floor-Mop Bucket - for the study. The room measures 6.5 by 4.5 metres and air-conditioned. The floor is covered with PVC tiles.

The Food Research Institute was initially established as a collaborative project between the Government of Ghana and the United Nations Development Programme in 1965. It was subsequently placed under the Council for Scientific and Industrial Research in 1970 at the expiration of the term of the joint project.

The mandate of the Food Research Institute (FRI) is to assist local food industries at all levels of organization to diversify and improve on their operations. Specifically, food product development, food preservation, nutrition studies, food analysis (Chemical & Microbiology) and agricultural economics.

(b) Noguchi Memorial Institute for Medical Research (NMIMR)

The NMIMR is part of the University of Ghana, Legon, and is located on the permanent site of the University of Ghana Medical School. The Institute is housed in a one storey permanent structure designed in the Japanese fashion and, indeed, built with grants donated by the Japanese Government. The rooms are centrally air-conditioned with standard laboratory facilities.

The Institute researches into tropical diseases and malnutrition. The identification of the bacterial isolates was carried out in the Bacteriology Laboratory of the Institute.

(c) Department of Botany - University of Ghana

The University of Ghana is located close to the Nogguchi Memorial Institute for Medical Research and about 16 km. from the coast. The Department is housed in a single storey reinforced concrete building with offices and teaching and research laboratories. Planning of the research procedure took place in the Department where the Science Laboratory Technician Training Scheme is organised.

3. MATERIALS

Eighty samples of in-use disinfectant solutions were taken over a period of one working week (15 - 19th February, 1993) at three-hour intervals each day. While the study was going on, the usual research activities of the laboratory included investigations on the microbial flora of cocoa products, fish meal and fermented maize meal, and Lyophilization of Lactic acid bacteria.

(a) Disinfectant

The disinfectant used in the study is phenolic with PCMX and DCMX as the active ingredients. The Rideal Walker Test Value is 4.2. The product is manufactured by S.C. JOHNSON WAX LTD., GHANA-ACCRA. Ten percent (v/v) solutions made with distilled water were prepared for the in-use test of all the selected sites. A trial test indicated the need to adjust the manufacturer's recommended concentration of 4% (v/v) to 10% (v/v) in order to compare with the Kelsey & Maurer (1966) and Maurer (1978) method.

(b) Diluent

Quarter Strength Ringer Solution with 3% Tween 80- Polyoxyethylene Sorbitan Monooleate as the Neutralizer.

The disinfectant was used for four sites:

i) Discard Jar
ii) Discard Culture Spreader Jar
iii) Discard Pipette Jar
iv) Floor-Mop Bucket.

(c) Discard Jar (Cylinder)

Two discard jars with capacity of 2,500 ml. each were used alternately. The open ends of the cylinders were covered with grease-proof paper firmly tied on before autoclaving at 121°C for 15 minutes. An amount of two litres of the disinfectant solution was prepared directly in the jar and the discard materials (microscope slides, Pasteur pipette & vials) put in soon after.

(d) Discard Culture Spreader Jar

An amount of 400ml of the in-use disinfectant solution was prepared in a sterile (autoclaved) 500ml capacity jar. Metallic spreaders used in spreading culture suspensions on agar surfaces were discarded into the jar.

(e) Discard Pipette Jar

Pipettes which had been used in transferring culture suspensions were placed in the in-use solution. An

amount of two litres of the disinfectant solution was put in the Discard Pipettes Jar soon after it had been autoclaved, and just before the pipettes were introduced.

(f) Floor-Mop Bucket

Two buckets (Galvanized & Plastic) were used alternately each other day. They were first autoclaved with the opening covered with paper which was held tight with Cellophane-tape. Two floor-mop heads, which were also used alternately, were wrapped in grease paper and also autoclaved. A bucket held five litres of the in-use solution.

The end of mop handle which was to be fixed to the mop was placed in 95% Alcohol for one hour before use. The mopping exercise was conducted by dipping the mop into the in-use disinfectant solution in the bucket and mopping the floor (PVC tiled) of the selected laboratory. The usual practice of the laboratory is to mop the floor first with detergent solution before mopping with a disinfectant solution. The same procedure was followed in this study.

A baseline microbial flora was determined for the floor by swabbing an area 10 x 10 cm with a sterile cotton swab and then rinsed in 9ml of sterile distilled water. The suspension was used to inoculate Nutrient Agar plates.

(g) Media

i) Nutrient Agar (NA)

Lab Lemco powder (oxoid)	-	1g
Yeast Extract (oxoid)	-	2g

Peptone (oxoid)	- 5g
Sodium chloride (BDH)	- 5g
Agar (oxoid)	- 15g
Distilled water	-1000ml.
(pH 7.4)	

ii) Potato Dextrose Agar (PDA) (DIFCO)

Potatoes, Infusion from	200g
Bacto - Dextrose	20g
Bacto - Agar	15g
Distilled water	1000ml.
(pH 5.6)	

iii) Quarter Strength Ringers Solution (BDH)

iv) Hugh & Leifson's medium

Peptone (oxoid)	- 2.0g
Sodium chloride (BDH)	- 5.0g
Dipotassium, Hydrogen Phosphate	- 0.3g
Basto-Agar	- 3.5g
Distilled Water	-1000ml

pH adjusted to 7.1 and 15ml of 0.2% bromothymol blue and sterile (filtered) dextrose to give 1.0% concentration.

v) Human Plasma

This was obtained by centrifuging 5ml of freshly withdrawn blood to deposit the cells and the supernatant plasma was decanted into a sterile screw-capped bottle.

PLATE 2: PHOTOGRAPH SHOWING FLOOR-MOP BUCKET AND MOP USED IN THE INVESTIGATION (X 1/20)

IN-USE TEST

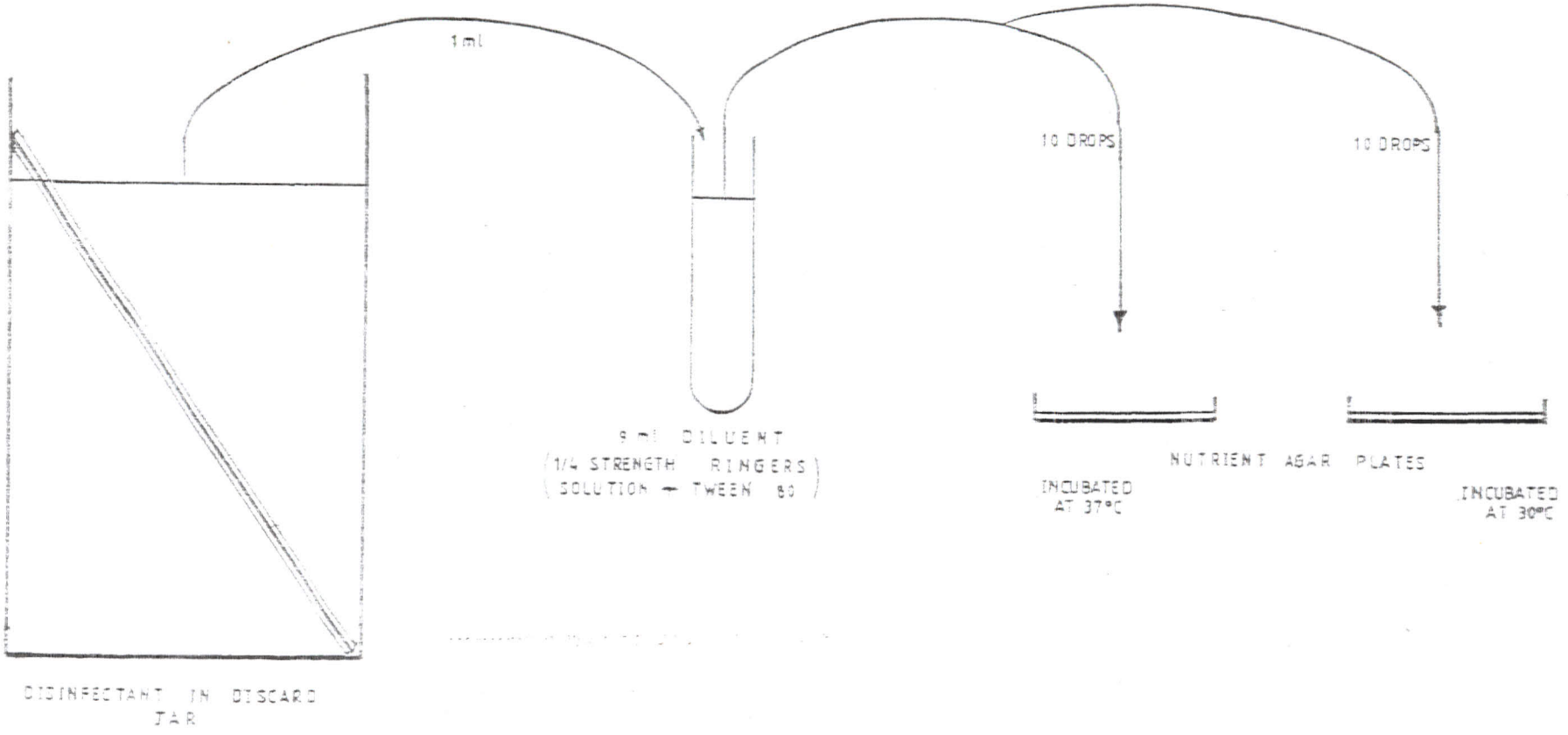


FIG. 1

4. METHODS

(a) Kelsey & Maurer In-Use Test

Test Requirements

Proximity of test sites and the nutrient agar plates allowed for the samples collected to be used within one hour of the addition of samples to the diluent. This is a requirement of the test (Maurer, 1970)

Diluent

In case there was an antimicrobial activity, nutrient broth diluent was also used in order to compare, with the quarter strength Ringer's solution with Tween 80 (neutraliser) diluent.

Sampling

A one millilitre sterile pipette with a pipette aid (Picoboy (R)) was used in taking one millilitre sample of the in-use disinfectant solution which was thoroughly mixed with 9ml sterile diluent (quarter strength Ringer's Solution plus Tween 80 -polyoxyethylene Sorbitan Moncoleate - neutraliser) in 25ml screw-capped bottle.

Inoculation

A sterile Pasteur pipette was used to withdraw a small volume of the disinfectant-diluent solution and 10 drops were separately and equidistantly placed on the surface of each of four well dried (at 55°C for 45 minutes) nutrient agar plates for each sample taken (Fig.1).

Two well dried surfaces of Potato dextrose agar were

also inoculated as above on the first day of the study.

IN-USE TEST

Incubation

The plates were incubated as follows:

1) Two plates of nutrient agar were incubated at 37°C for 48 hours

2) Two plates of nutrient agar were incubated at 30°C (room temperature) for 48 hours.

3) To plates of Potato dextrose agar were incubated at 30°C for 5 days.

After Incubation

After incubation, the plates were examined for growth and determination of colony forming units/ml. Single colonies were picked from the nutrient agar plates and sub-cultured by streaking on a set of dried nutrient agar plates and incubated at 37°C overnight to obtain discrete colonies.

(b) Gram Reaction (Collins & Lyne, 1984)

Discrete colonies were picked and stained with Gram's stain. Using clean grease-free microscope slides, Smears were first prepared and air-dried before fixing cover a bunse burner flame.

The film was stained with methyl violet (0.5g/100ml distilled water) for 20 seconds before washing off and replacing with iodine (2g potassium iodide and 1g iodine

- ground together and made up to 100mls. distilled water) for one minute. The iodine was washed off with 95% alcohol. It was finally washed with water and counterstained with Safranin for 1 minute. The slides were dried at 35°C for 15 minutes and observed under the light microscope using x100 objective (oil immersion).

The morphology (colony morphology, cell morphology and arrangement of cells) and Gram reaction are recorded in Table 4.

(c) Biochemical Tests

Biochemical tests were performed for some of the isolates based on the information provided by the microscopic examination.

i) Catalase Test (Cruickshank, 1968)

The test was conducted by picking small amount of the culture (two colonies) from a nutrient agar plate using a sterile loop and inserting into a hydrogen peroxide solution (3%) held in a small clean tube to observe gas bubble production.

ii) Oxidase Test (Merck, 1992)

Test strips - Bactident(R) (Merck), oxidase were used for the oxidase test.

iii) Hugh and Leifson (oxidative-fermentative) test (Collins & Lyne, 1984)

Two tubes (for each isolate) containing 5ml of

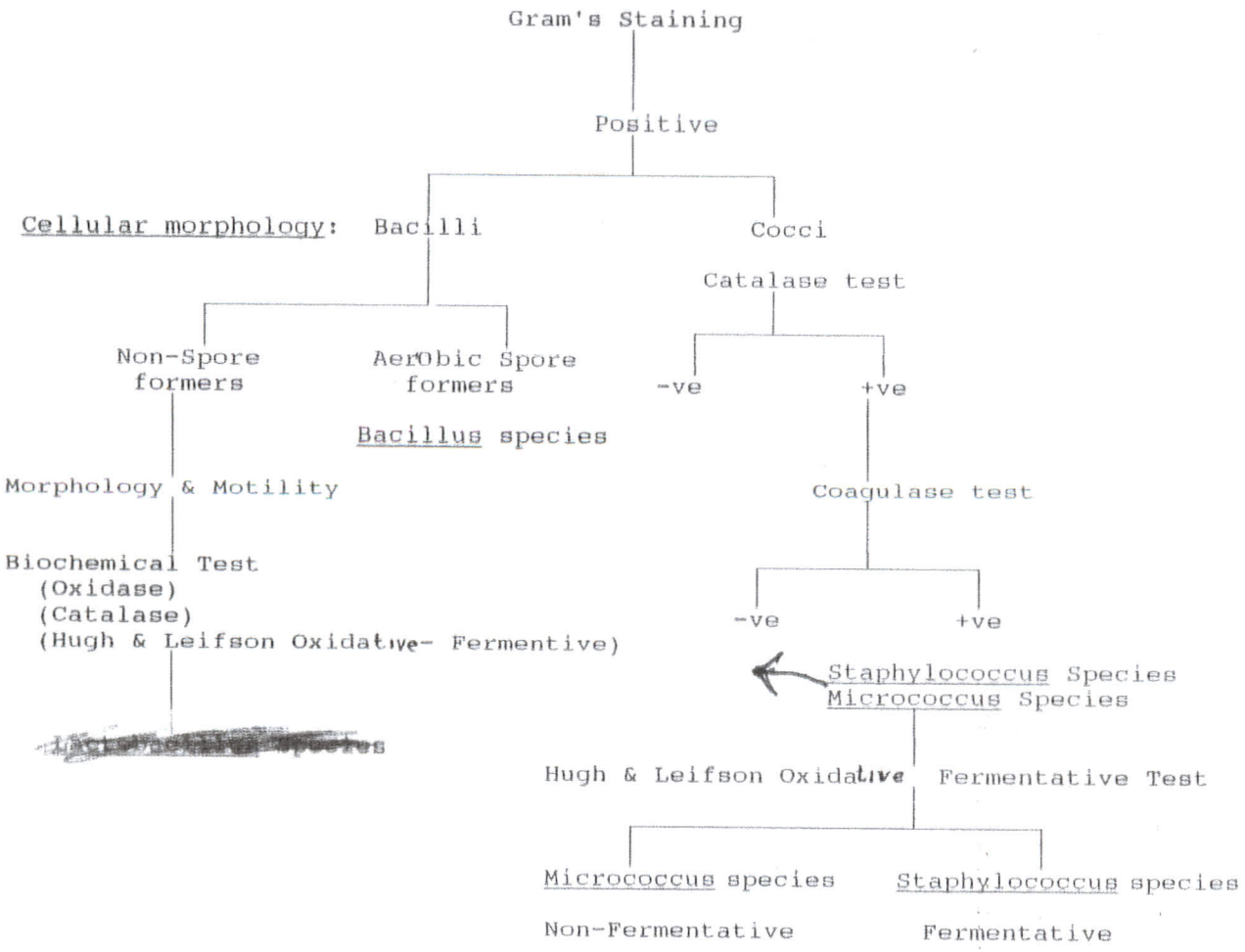
sterile Hugh and Leifson's medium were heated in boiling water for 10 minutes to drive off oxygen. They were cooled and inoculated with the test organism. One of the tubes was incubated anaerobically by sealing off the surface with 2cm thick sterile parafin oil for 72 hours and the other left uncovered. The change of indicator (from blue to yellow) due to acid production as a result of either oxidative or fermentative metabolism was noted and recorded.

iv) Slide Coagulase Test (Collins & Lyne, 1984)

The fermentative action of the Gram positive cocci suggested that they might be staphylococci and not micrococci. Two colonies were emulsified in a drop of sterile water on a clean microscope slide and observed for 20 seconds for signs of clumping.

When no clumping occurred, a sterile straight wire loop was dipped into human plasma and stirred in the bacterial suspension. The result is negative if no agglutination occurred.
Fig.3 illustrate the identificational scheme followed.

FIG. 2 IDENTIFICATION SCHEME FOLLOWED



CHAPTER FOUR

RESULTS

The results obtained with the Nutrient Agar Plates are set out in Table 1, 2 and 3 and the identification scheme followed are illustrated in Fig.2 and 3. The colonial morphology of the various cultures were observed and noted. The two diluents, namely, the quarter strength ringer's Solution plus Tween 80 (neutralizer and the nutrient broth, were found to produce the same results for all the trials. Furthermore, the Colony Forming Units/ml were found to be the same for the two incubation temperatures (30°C and 37°C).

Table 1: Bacterial Counts of Nutrient Agar (NA) Plates of In-use Test of Disinfectant (Crusade) samples at Four Different Sites Taken at Different Intervals

WORKING DAY	SITE	TIME SAMPLE WERE TAKEN AFTER INTRODUCTION OF MATERIAL (HR)	CFU/ML ON INCUBATED NA AT 30°C	CFU/ML ON INCUBATED NA AT 37°C
MONDAY 15/2/93	DISCARD JAR	3	1,800	1,800
		6	850	850
		9	400	400
		24	150	150
	SPREADER JAR	3	250	250
		6	100	100
		9	50	50
		24	0	0
	PIPETTE JAR	3	400	400
		6	200	200
		9	100	100
		24	50	50
	FLOOR MOP BUCKET	3	7,000	7,000
		6	3,500	3,500
		9	1,500	1,500
		24	500	500
TUESDAY 16/2/93	DISCARD JAR	3	2,500	2,500
		6	1,200	1,200
		9	600	600
		24	200	200
	SPREADER JAR	3	300	300
		6	150	150
		9	50	50
		24	0	0
	PIPETTE JAR	3	350	350
		6	150	150
		9	50	50
		24	0	0
	FLOOR MOP BUCKET	3	5,000	5,000
		6	2,600	2,600
		9	1,200	1.200
		24	450	450

Table 1: Bacterial Counts of Nutrient Agar (NA) Plates of In-use Test of Disinfectant (Crusade) samples at Four Different Sites Taken at Different Intervals. (contd.)

WORKING DAY	SITE	TIME SAMPLE WERE TAKEN AFTER INTRODUCTION	CFU/ML ON INCUBATED NA AT 30°C	CFU/ML ON INCUBATED NA AT 37°C

		OF MATERIAL (HR)		
		3	1,200	1,200
	DISCARD	6	550	550
	JAR	9	200	200
		24	50	50
WEDNESDAY				
	SPREADER	3	200	200
	JAR	6	100	100
		9	50	50
17/2/93		24	0	0
	PIPETTE	3	250	250
	JAR	6	100	100
		9	50	50
		24	0	0
	FLOOR	3	4.000	4,000
	MOP	6	2,000	2,000
	BUCKET	9	900	900
		24	350	350
		3	1,200	1,200
	DISCARD	6	550	550
	JAR	9	200	200
		24	50	50
THURSDAY				
	SPREADER	3	200	200
	JAR	6	100	100
		9	50	50
18/2/93		24	0	0
	PIPETTE	3	250	250
	JAR	6	100	100
		9	50	50
		24	0	0
	FLOOR	3	4,000	4,000
	MOP	6	2,000	2,000
	BUCKET	9	900	900
		24	350	350

Table 1: Bacterial Counts of Nutrient Agar (NA) Plates of In-use Test of Disinfectant (Crusade) samples at Four Different Sites Taken at Different Intervals. (contd.)

WORKING DAY	SITE	TIME SAMPLE WERE TAKEN AFTER INTRODUCTION OF MATERIAL (HR)	CFU/ML ON INCUBATED NA AT 30°C	CFU/ML ON INCUBATED NA AT 37°C
		3	1,500	1,500
	DISCARD	6	700	700
	JAR	9	300	300
		24	100	100
FRIDAY				
	SPREADER	3	300	300

	JAR	6	150	150
		9	50	50
19/2/93		24	0	0
	PIPETTE	3	350	350
	JAR	6	150	150
		9	50	50
		24	0	0
	FLOOR	3	2,500	2,500
	MOP	6	1,200	1,200
	BUCKET	9	650	650
		24	200	200

TABLE 2: AVERAGE NUMBER OF COLONY FORMING UNITS/ML (CFU/ML) OF SURVIVING BACTERIA IN IN-USE DISINFECTANT SOLUTIONS FOR DIFFERENT SITES

TIME SAMPLE WAS TAKEN AFTER INTRODUCTION OF MATERIAL (HR)	SITE & CFU/ML			
	A	B	C	D
3	1,800 (us)	200 (us)	310 (us)	4,300 (us)
6	850 (us)	130 (s)	140 (s)	2,160 (us)
9	400 (us)	50 (s)	60 (s)	1,010 (us)
24	150 (s)	0 (s)	0 (s)	360 (us)

A: Discard Jar

B: Discard Spreader Jar

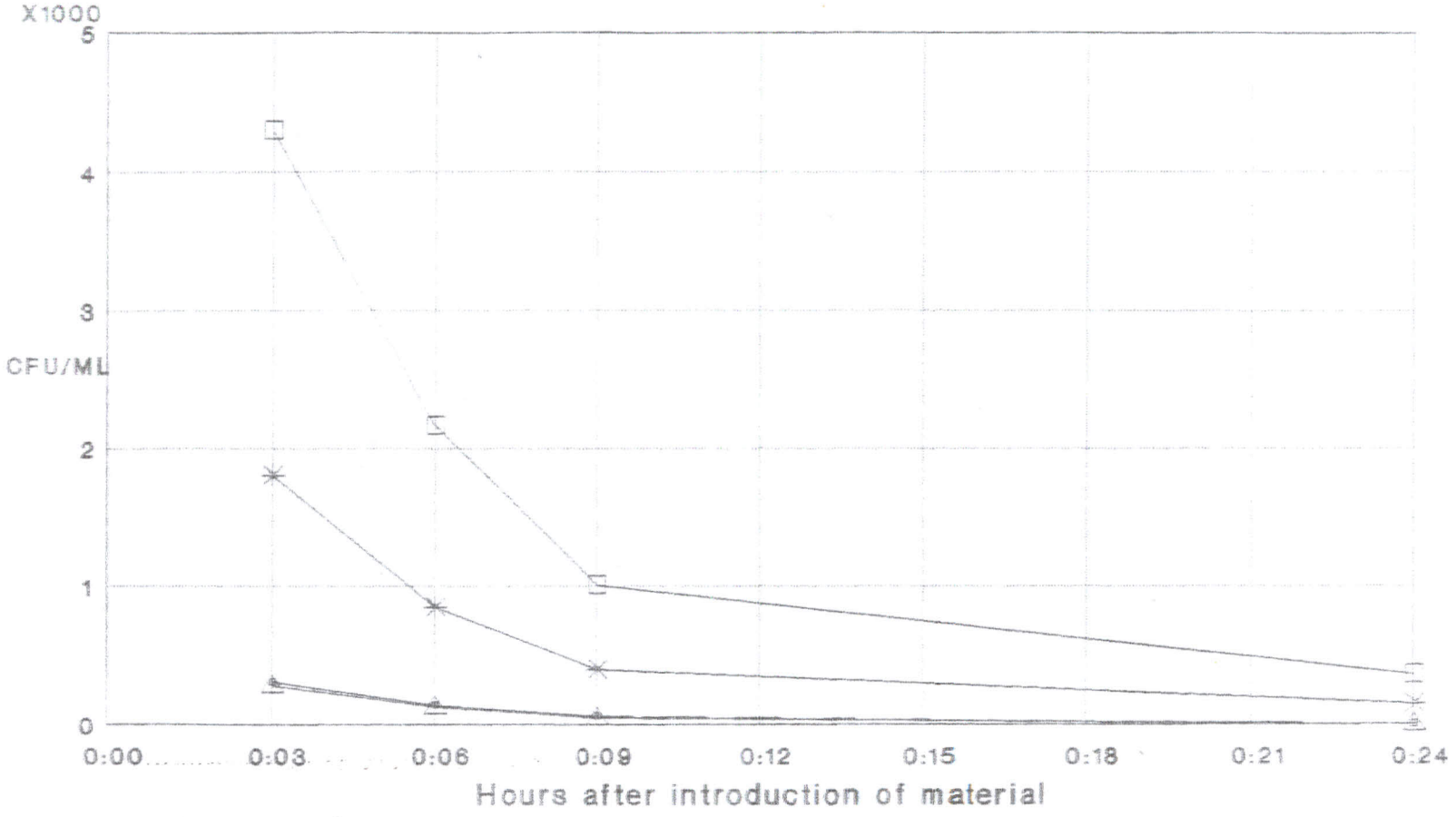
C: Discard Pipette Jar

D: Floor Mop Bucker

(s): Satisfactory)
(us): Unsatisfactory)

Kelsey & Maurer Interpretation based on CFU/ml (less than 250 CFU/ML is satisfactory and greater than 250 CFU/ML is Unsatisfactory.

FIG. 3. AVERAGE BACTERIAL SURVIVAL
IN IN-USE DISINFECTANT SOLUTIONS



CFU: Colony Forming Units

PLATE 3: PHOTOGRAPH SHOWING GROWTH OF BACTERIAL FROM FLOOR-MOP BUCKET ON NUTRIENT AGAR PLATE (X 11/30)

FROM LEFT: 3,6,9 & 24 HOURS OF EXPOSURE, RESPECTIVELY

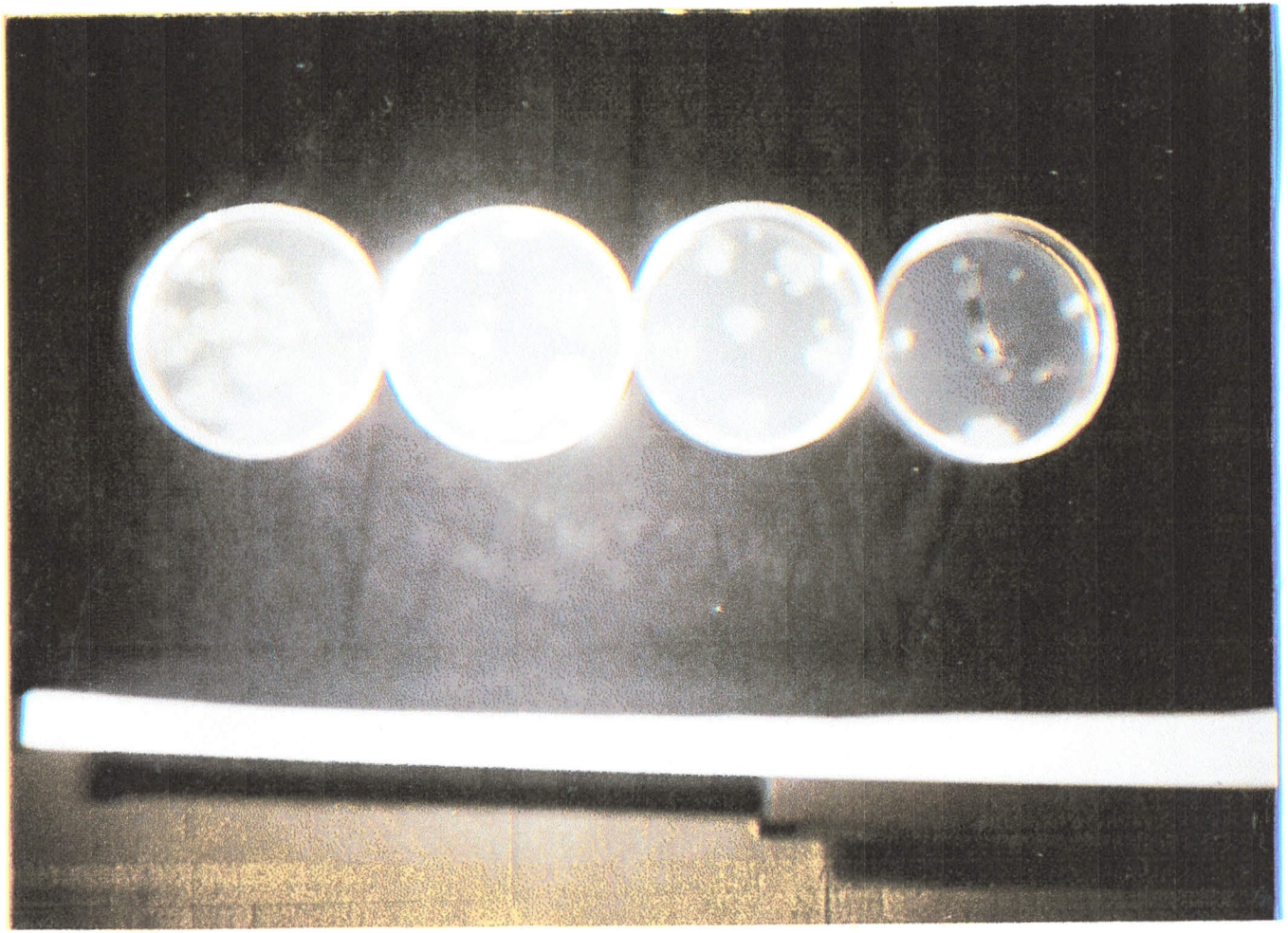


TABLE 3: COLONIAL MORPHOLOGY AND GRAM REACTION ON NUTRIENT AGAR

ISOLATE CODE	COLONIAL MORPHOLOGY	GRAM REACTION	FORM & ARRANGEMENT	ORGANISM (PRESUMPTIVE)
1DJ	4mm, flat & rhizoid	Positive	Sporing rods	Bacillus species
2SJ	1mm, entire, transluscent & Moist	" "	Pleomorphic Non-sporing rods	Lactabacillus species
1PJ	4mm, entire & mucold	"	Rods	Bacillus species
1FMB	4mm, irregular flat & dry	"	Sporing rods	Bacillus species
2FMB	1.5mm, yellow entire smooth & moist	"	Cocci in clusters	Staphylococci) Micrococci)species
1BL	4mm, flat & rhizoid	"	Sporing rods	Bacillus species
2BL	1.5mm, yellow entire smooth & moist	"	Cocci in clusters	Staphylococci) Micrococci)species

D: Discard Jar; SJ: Spreader Jar; PJ: Pipette Jar; FMB: Floor mop bucket

BL: Baseline

TABLE 4: BIOCHEMICAL REACTIONS OF TWO ISOLATES

ISOLATE	MICROSCOPIC DESCRIPTION	GRAM	CATALASE	OXIDASE	COAGLASE	OXIDATIVE FERMENTATIVE	ORGANISM (DEFINITIVE)
2DJ	Pleomorphic Non-sporing rods	Positive	Negative	Negative		Fermentative	Lactabacillus species
2FMP	Cocci in clusters	-	Positive	"	Negative	"	Non Pathogenic Staphylococcus species

Despite the variations in terms of bacterial numbers, the pattern of bacterial survival as illustrated in Fig.3 is common to all the sites, that is,thelonger the time of exposure the lower the count.

The results also showed that there was about 50% subsequent reduction of bacterial population at the three-hourly interval (3-6-9 hours). Considering all the sampling times together, seven out of 16 of the viable counts were deemed satisfactory according to the Kelsey & Maurer assessment standard - (If CFU/ML is less than 250 then the performance of the in-use solution is satisfactory. If the CFU/ML is greater than 250 then the performance of the in-use solution is unsatisfactory).

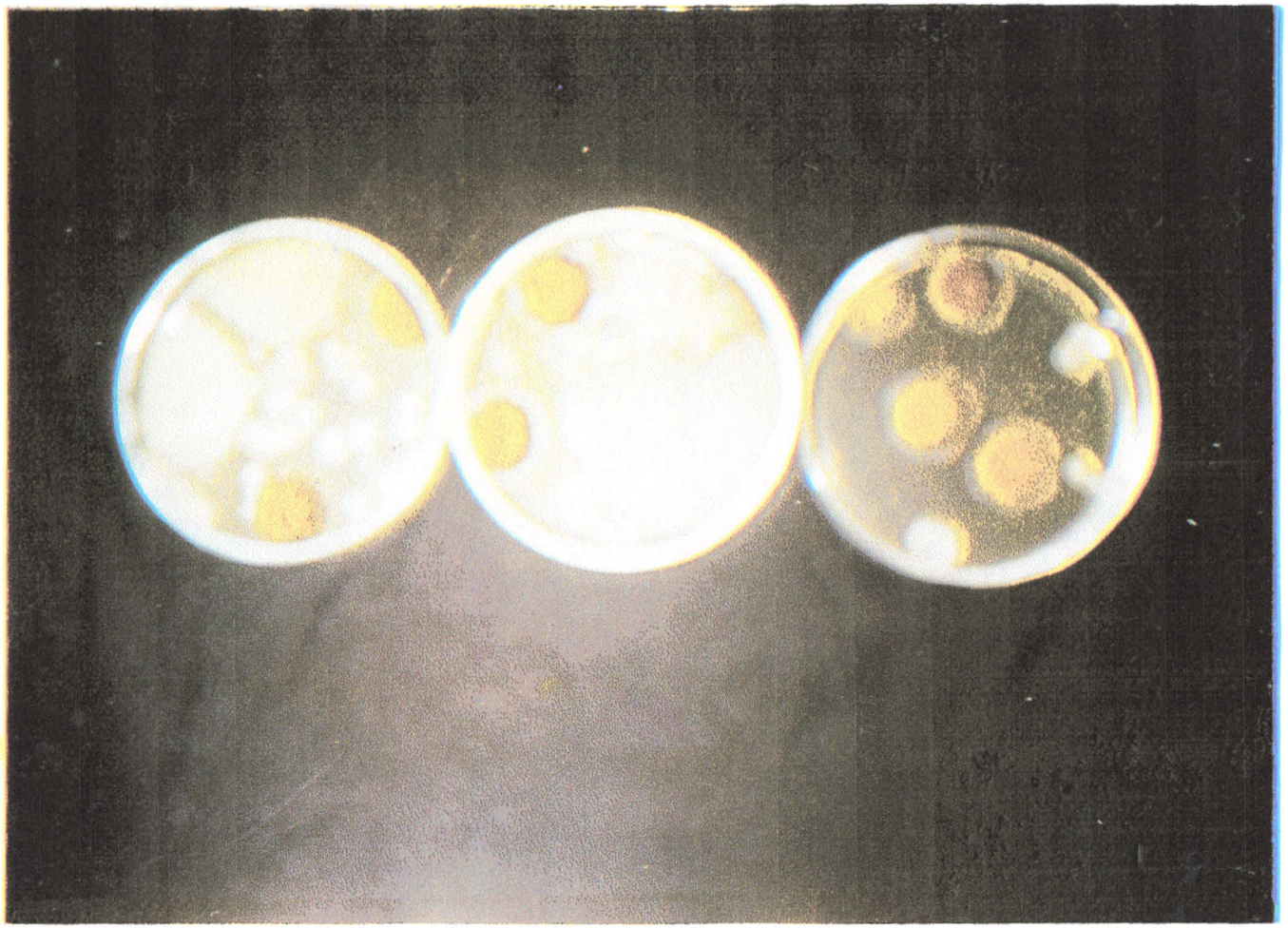
Aspergillus niger and Penicillium species were isolated from the first sample (3 hours) of the Discard Jar (Table 5, Plate 4).

TABLE 5: MICRO-ORGANISMS ISOLATED FROM SITES OVER THE WORKING WEEK (15TH-19TH FEBRUARY, 1993)

SITE	ORGANISMS
DISCARD JAR	Gram positive aerobic Bacillus species, Gram positive aerobic & sporing Bacillus species Lactobacillus species Penicillium species Aspergillus species
DISCARD SPREADER JAR	Gram positive aerobic Bacillus species
DISCARD PIPETTE JAR	Gram positive aerobic Bacillus species
FLOOR MOP BUCKET	Gram positive aerobic Bacillus species Gram positive aerobic & sporing Bacillus species Coagulase - negative Staphyloccus
BASELINE (FLOOR)	Gram positive aerobic sporing Bacillus species Gram positive aerobic Bacillus species Staphylococcus albus

PLATE 4: PHOTOGRAPH SHOWING GROWTH OF FUNGI FROM THE DISCARD JAR ON POTATO DEXTROSE AGAR (X 5/9)

FROM LEFT: AFTER 3, 6 AND 9 HOURS OF EXPOSURE, RESPECTIVELY



CHAPTER FIVE

DISCUSSION

The choice of exposure time in such a study is quite essential. Kelsey and Maurer (1966) simple in-use test involves sampling at the end of the day (ie. 9 hours used in this study). Collins and Lyne (1984) have 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.

The initial bacterial loads of the materials of this investigation apparently varied widely. For, on the first sampling after three hours, the number of bacterial surviving in the disinfectant solutions of the Discard Jar and the Floor-Mop Bucket was extremely high (see Tables 1 and 2). After nine hours, the surviving population levels were still unacceptable high. It took 24 hours to reduce the population in the Discard Jar to satisfactory. By the standards of both Kelsey and Maurer (1966) and Collins and Lyne (1984), the disinfectant used in this investigation (Crusade$^{(R)}$) was unable to disinfect the Floor-mop satisfactorily, even though an unusually high concentration of 10% (v/v) was used.

It will always be difficult to estimate even roughly the bacterial load being introduced into a discard jar containing the in-use disinfectant solution. It is therefore essential that the longest possible exposure time within 24 hours should be chosen so that the hazard of a large percentage of the microorganisms surviving should not occur. The microbiology laboratory would have to adopt this suggestion if it would continue to use this particular disinfectant (Crusade$^{(R)}$).

The brand of phenolic disinfectant (Crusade$^{(R)}$), with Rideal-Walker Test Value 4.2 claim was selected because it is locally manufactured and readily available. It must also be admitted that promotional adverts had influence on the choice.

Christensen et al.(1982) used a 2% (v/v)concentration of another phenolic disinfectant (Bacillotox$^{(R)}$) compound in their in-use tests. The concentration of Crusade$^{(R)}$ used in this investigation was, therefore five times higher. This implies a greater expenditure for the Food Research Institute as large quantities of disinfectant are used routinely. It is desirable to examine the efficiency of other locally available disinfectants and decide finally on a choice, based on efficiency and costs.

CHAPTER SIX

SAFETY MEASURES

Safety, pertaining to general laboratory practice was maintained throughout the exercise.

1. Laboratory coat was worn at all times.

2. Bench tops were kept neat and clean and in addition decontaminated with 70% alcohol before and after the use of the bench for work.

3. Disposable gloves were worn in all the procedures - pipetting, plating, discarding, etc.

4. A pipette aid (picoboy$^{(R)}$), was used in effecting transfer of liquid cultures at all stages.

5. Inoculating loops and straight wires were sterilized in a hooded Bunsen burner flame to prevent unsterilized Culture Spurting from the loop or wire on the bench.

6. The disinfectant solutions were prepared using protective barriers such as goggles for the eyes, mask for the respiratory tract and disposable gloves to protect the skin from accidental splashes.

7. Disposal of discarded materials (cultures, Pasteur pipettes, transfer pipettes, etc.) were effected by autoclaving first before those to be recycled were cleaned.

8. The hands were washed with soap before leaving the laboratory on any occasion.

CHAPTER SEVEN

CONCLUSION AND RECOMMENDATION

The study showed that testing of in-use disinfectant solutions is possible in ordinary laboratories where sites used in this investigation and other similar ones are present. The test must, therefore, not be limited to hospitals alone as previous publications seem to indicate.

In this connection, it is recommended that:

1. In-use test must be conducted at least once a month from all sites to establish the effectiveness of the disinfectant being used in the laboratory.

2. Other disinfectants available in the country should be tested, so that a substitute, if costs permit, should be adopted.

3. Since the disinfectant solutions are discarded after 24 hours, used materials introduced into the sites should be allowed to remain in the disinfectant solutions for the possible maximum time to allow for the destruction of the microorganisms.

CHAPTER EIGHT

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