IIRT
Institute for Industrial Research & Toxicology
औद्योगिक अनुसंधान एवं विष विज्ञान संस्थान
Registration No. 1303/C/CPCSEA (Ministry of Environment & Forests, Government of India)
License No.: UP37370000001 (Food and Drug Administration, UP)
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GLP Certified, NABL (ISO/IEC 17025) Accredited

Virosil

VALIDATION REPORT

FOR

DISINFECTANT VALIDATION STUDY

Study Sponsored by: Sanosil Biotech Pvt. Ltd.

Study Conducted at: Indian Institute of Toxicological Research

REPORT APPROVED ON	**02-02-2024**

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1.0 REPORT APPROVALS

Signing of this approval page of Report for disinfectant validation that this document has been prepared, reviewed & approved by the following functional department. If any modification in the Report becomes necessary and addendum shall be prepared, reviewed and approved.

PREPARED BY:

Function	Name	Designation	Signature	Date
Microbiology	Tanuja	Microbiologist	Tanuja	01-02-2024

REVIEWED BY:

Function	Name	Designation	Signature	Date
Microbiology	George Hershel	Sr. microbiologist		02-02-2024
Microbiology	Anshul Mishra	Tech. Manager		02-02-2024
Quality Assurance	Divya Jain	QA Manager		02-02-2024

APPROVED BY:

Function	Name	Designation	Signature	Date
Quality Assurance	Shalini Mishra	QA Head		02-02-2024

2.0 OBJECTIVE

Objective of this report is to evaluate the efficacy of disinfectant solution which will be used for the sanitization in controlled area.

3.0 SCOPE

The scope of this study is applicable for Virosil 5 % and Microbial Strain applicable as per protocol.

4.0 RESPONSIBILITY

Responsibilities of individual functional areas are as follows:

Quality Control	➢ To prepare the report ➢ Analysis of the samples as per the defined procedure ➢ Summarize the analytical data
Quality Assurance	➢ Review and Approval report ➢ Implementation of report ➢ Approval of any Report amendments, if necessary

5.0 TRAINING

Training was imparted to familiarize the trainee with validation protocol for disinfectant validation study. Training was recorded as per Annexure-I.

6.0 DESCRIPTION OF THE PRODUCT:

Disinfectant: Sample received at site on 13/01/2024 and shall be Store at room temperature.

S. No.	Name of Sample	Batch No.	Date of Mfg.	Date of Exp.	Composition
1	Virosil	VBS23242	12/2023	11/2025	● Hydrogen Peroxide-10%. ● Diluted Silver nitrate Solution-0.01%.

Surface Templates: - Following standard surface templates of size 5cm X 5cm ($25cm^2$) as given below are used to execute the study.

S. No.	Disinfectant Use	Type of Template	Representative Surface
1.	Virosil	Epoxy	Floor
2.		Glass	Window, View Panel
3.		Kota stone	Working bench
4.		Stainless Steel (S.S.316)	Equipment Surface

7.0 Microbial Cultures: Following standard microbial cultures are used to execute the study and environment isolate used to check the efficacy on different surfaces only.

S. No.	Name of Cultures
1.	*Pseudomonas aeruginosa* ATCC/NCIM 9027/2200(Gram negative small rods vegetative microorganism)
2.	*Staphylococcus aureus* ATCC/NCIM 6538/2079 (Gram positive vegetative microorganism)
3.	*Bacillus subtilis* ATCC/NCIM 6633/2063 (Spore forming microorganism)
4.	*Candida albicans* ATCC/NCIM 10231/3471 (Yeast)
5.	*Aspergillus brasiliensis* ATCC/NCIM 16404/1196 (Mould)
6.	*Ecoli* ATCC/NCIM 8739/2065 (Gram negative rod shaped)

7.1 Media/Reagents: Following Media/Reagents used to execute the study:

- Soyabean Casein Digest Agar
- De-Engley Neutralizer Broth
- 0.1 % Peptone Water
- 0.9% NaCl Solution

7.2 Equipment's/Accessories: Following equipment/accessories are used to execute the study:

- Laminar Air Flow
- BOD Incubator (20-25°C)
- BOD Incubator (30-35°C)
- Refrigerator (2-8°C)
- Micropipette
- Sterile Micro tip
- Vortex Mixer
- Vacuum Pump
- Sterile Spreader
- Sterile Petri plates

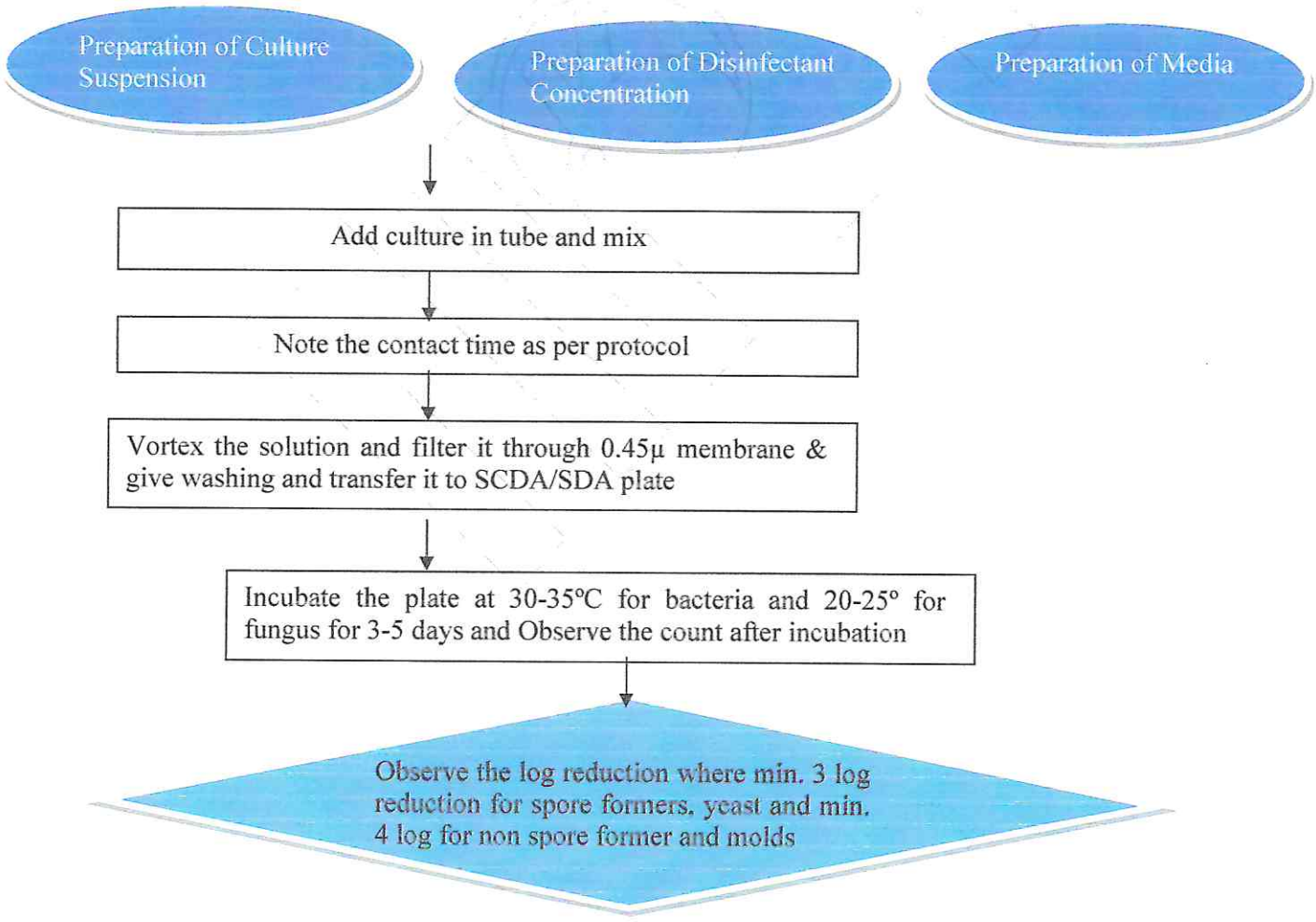
- Sterile Forceps
- 0.45µ Sterile Membrane
- Filtration Assembly
- Surface Templates

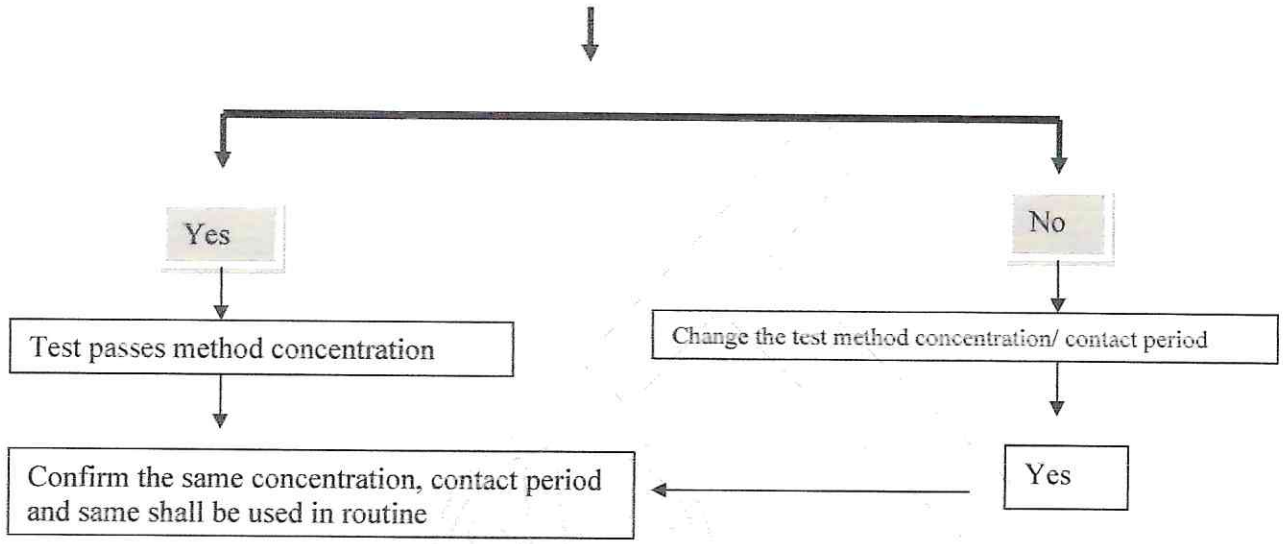
8.0 VALIDATION PROCEDURE

The validation procedure for disinfectant efficacy is carried out in two parts:

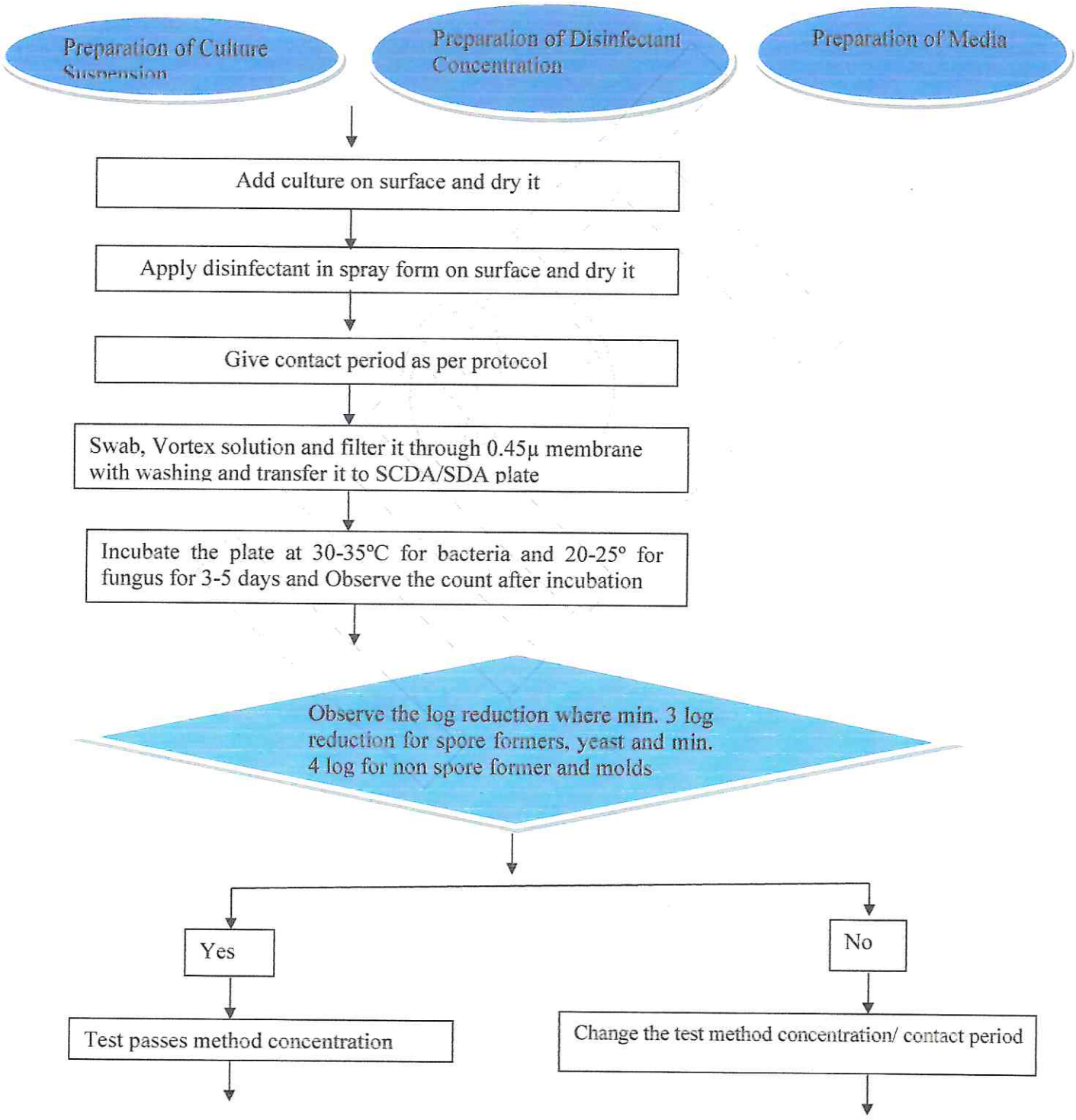
1. **Use dilution test:** To determine the effective concentration and to screen the use concentration at various contact times.
2. **Surface challenge test or Surface recovery test:** To determine the effectiveness of use concentration in reducing the bioburden on the surface.

Flow Diagram for Use Dilution test method




Yes
No
Test passes method concentration
Change the test method concentration/ contact period
Yes
Confirm the same concentration, contact period and same shall be used in routine

Flow diagram for Surface contact method



Confirm the same concentration, contact period and same shall be used in routine ← Yes

Day- 1st Activity

9.0 Preparation of Culture Suspension:

9.1 Transfer aseptically one loop full culture in 10 ml sterile 0.9% normal saline solution under LAF.

9.2 Close the tube and vortex vigorously the contents for about five minutes. This constitute dilution is consider as stock suspension.

9.3 Take 1 ml from stock suspension tube and inoculate into the tube containing 9 ml sterile 0.9% normal saline solution under LAF. This constitute dilution is considered as 10^{-1}.

9.4 Repeat the procedure as 10.2 to 10.4 and prepare the dilution as 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} & 10^{-7}.

9.5 Inoculate 1 ml culture suspension from tube no. 10^{-5}, 10^{-6} and 10^{-7} into plates in duplicate by pour plate method under LAF.

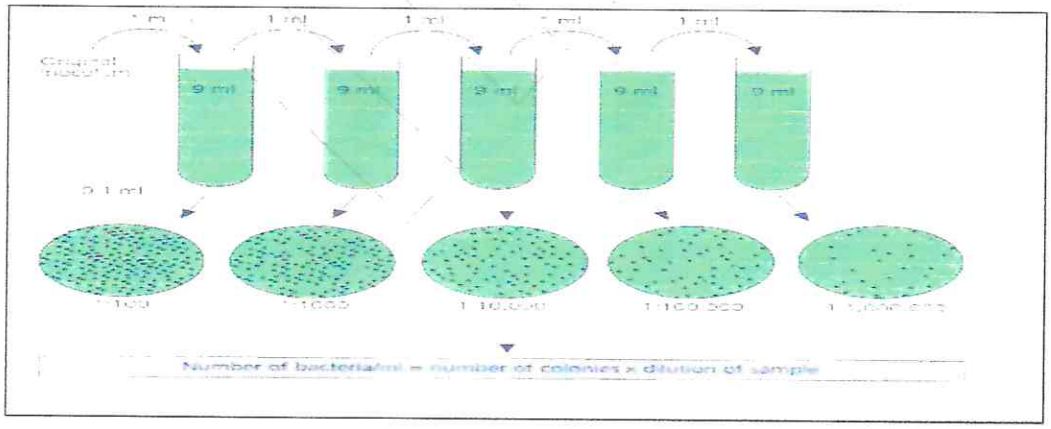
9.6 After inoculation of 1 ml culture suspension into plates, pour the 20-25 ml media cooled to 45-50°C in the petriplates.

9.7 Incubate the plates or tubes at 30-35 °C for 48-72 hrs. for bacterial growth.

9.8 Incubate the plates or tubes at 20-25 °C for 72-120 hrs. for fungal growth.

9.9 After completion of incubation period count the colonies.

9.10 If sufficient growth is not observed, repeat the process of preparation of culture suspension.



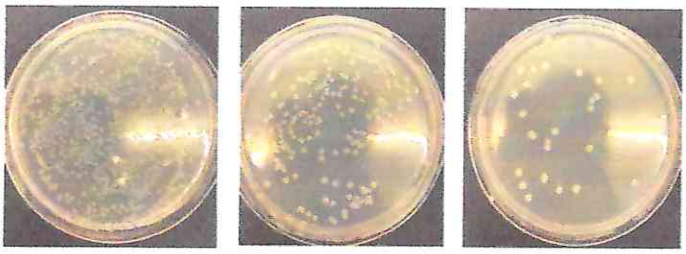
Day- 5th Activity

Date of Stock Suspension Preparation	16-01-2024
Date of Serial Dilution	16-01-2024
Date of Observation	19-01-2024 / 22-01-2024

Name of Culture	Incubation temp./Time	Date of test	Date of Observation	Dilution tested	Observed cfu
Pseudomonas aeruginosa	30-35°C/ 72hrs.	16-01-2024	19-01-2024	10^{-5}	75
				10^{-6}	19
				10^{-7}	5
Staphylococcus aureus	30-35°C/ 72hrs.	16-01-2024	19-01-2024	10^{-5}	62
				10^{-6}	22
				10^{-7}	2
Bacillus subtilis	30-35°C/ 72hrs.	16-01-2024	19-01-2024	10^{-5}	84
				10^{-6}	20
				10^{-7}	3
E.coli	30-35°C/ 72hrs.	16-01-2024	19-01-2024	10^{-5}	72
				10^{-6}	28
				10^{-7}	5
Candida albicans	20-25°C/ 5 days	16-01-2024	22-01-2024	10^{-5}	66
				10^{-6}	18
				10^{-7}	4
Aspergillus brasiliensis	20-25°C/ 5 days	16-01-2024	22-01-2024	10^{-5}	58
				10^{-6}	14
				10^{-7}	2

Some observation of results after serial dilution:

S.aureus



C.albicans

10.0 Preparation of Disinfectants: Prepare the disinfectants of 5% concentration as per below mentioned procedure.

- Take clean and dry 1000 ml measuring cylinder.

- Take 5 ml Virosil disinfectant.
- Dilute with 950 ml purified water to make the volume 1000 ml mix uniformly.
- Label the container and keep it in separate area.
- Prepare the disinfectant and consume in same day.

S. No.	Name of Disinfectant	Recommended Concentration by the Manufacturer	Date of Preparation	Concentration Used for Validation
1	Virosil	5 %	23-01-2024 & 24-01-2024	5 %

11.0 Determination of disinfectants concentration and contact time: -

11.1. Perform the activity of disinfectant efficacy test at one concentration (recommended by manufacturer) with three different contact time.

11.2. Transfer 10 ml of each concentration of disinfectant into separate sterile test tubes.

11.3. Inoculate each disinfectant test tube with 0.1ml of all listed culture having a microbial count at 10^{-3}.

11.4. Note the time after addition of the culture suspension in disinfectant test tube.

11.5. Filter all the disinfectant preparations separately at 0, 5, and 15 minutes time interval by membrane filtration method.

11.6. For filtration of disinfectants preparations perform pre-wetting of 0.45µm size membrane filter with 100 ml of sterile De-Engley Neutralizer Broth.

11.7. Filter 10 ml of disinfectant preparation through 0.45µm size membrane filter.

11.8. Rinse the membrane filter of 0.45µm size by 3x100 ml of De-Engley Neutralizer Broth.

11.9. Make sure that no sample is present in the filtration cup on and transfer the membrane filter aseptically on to the Pre-incubated SCDA plate.

11.10. Positive Control: Pre-wet the membrane filter with 100 ml of sterile 0.1 % peptone water. Rinse the membrane with 3×100 ml of sterile De-Engley Neutralizer Broth. In the final rinsing add 0.1 ml of each listed culture suspension having count not more than 100 CFU and filter through 0.45µm size membrane filter. Make sure that no sample is present in the

filtration cup on and transfer the membrane filter aseptically on to the In-house Pre-incubated SCDA plate.

11.11. Negative Control: Perform pre-wetting of 0.45µm size membrane filter with 100 ml sterile 0.1 % peptone water. Rinse the membrane with 3x100 ml of sterile De-Engley Neutralizer Broth.

11.12. Make sure that no sample is present in the filtration cup on and transfer the membrane filter aseptically on to the pre-incubated SCDA plate.

11.13. Media Negative Control: Keep one pre-incubated SCDA plate as media negative control.

11.14. Incubate positive control of bacterial cultures at 30-35°C for 3 days and for fungal at 20-25°C for 5 days.

11.15. Incubate all the test sample, negative control and media negative control for bacterial cultures at 30-35°C for 3 days and for fungal at 20-25°C for 5 days.

11.16. After incubation completion observe the results and report the results in validation report.

Note: - Based on the above study, established contact time and concentration of the disinfectant efficacy will be verified for disinfectant efficacy by surface swab method.

Day-6th Activity

12.0 Determination of Disinfectant Efficacy by Surface Swab Method.

12.1. Perform the activity of disinfectant efficacy test with the previously determined concentrations and contact time by using all surface templates.

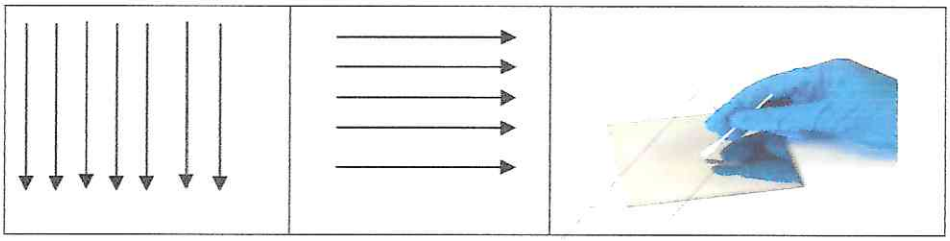
12.2. Aseptically, transfer all required standard surface template of size 5cm × 5cm (25cm^2) and accessories in LAF to start disinfectant efficacy of disinfectant.

12.3. Clean the surface template by 70 % v/v IPA. After that clean the surface template by sterile purified water.

12.4. Add 1ml of culture suspension of *Escherichia coli* containing (1000-100000 CFU) and spread it evenly on surface template with in the size of 5cm × 5cm (25cm^2).

12.5. After adding culture suspension allow the surface template to dry.

Sampling interval and Sampling type for Virosil are as per below mentioned table:



Horizontal Swabbing & Vertical Swabbing

Prepared Disinfectant Concentration	**Sampling Interval Time**
5 %	After 0 minute
	After 5 minute
	After 15 minute

12.6. Evenly spread selected concentration of disinfectant on the surface templates with the help of sterile mop/spreader.

12.7. After drying of surface of template, apply the sufficient quantity of disinfectant of known concentration i.e 5 % on the template surface.

12.8. Collect the swab sample from selected area of 5cm × 5cm ($25cm^2$) with the help of sterile cotton swab by fallowing the below mentioned table.

12.9. Put the swab sample into 10 ml of sterile 0.9 % normal saline tube.

12.10. Vortex the tube to mix the suspension uniformly.

12.11. Pre wet the membrane filter having a nominal pore size of 0.45 µ using 100 ml of sterile De-Engley Neutralizer Broth through the filtration cup.

12.12. Immediately transfer the whole content of swab sample tube into the filtration cup and filter the sample through 0.45µ membrane filter. Rinse the membrane filter with 3 × 100 ml of De-Engley Neutralizer Broth. Make sure that no sample is present in the filtration cup on and transfer the membrane filter aseptically on to the Pre-incubated SCDA plate.

12.13. Repeat the same method as described in 12.2.1 to 12.2.12 for (*Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus brasiliensis)* each bacterial culture and fungal culture.

12.14. **Positive control:** Perform the procedure from 12.2.2 to 12.2.12 without applying disinfectant for all challenged organisms separately.

12.15. **Negative control:**

(a) **Process Negative Control:** Perform the procedure from 12.2.2 to 12.2.12 with applying neither disinfectant nor challenged organisms on surface template separately.

(b) **Media negative control:** Keep one Pre-incubated SCDA plate as media negative control.

12.16. **Incubation and Results observation:**

- Iincubate the test sample, process negative control and media negative control for bacterial cultures at 30-35°C for 3 days and incubate test sample, process negative control and media negative control for fungal cultures at 20-25°C for 5 days.
- Incubate positive control of bacterial cultures at 30-35°C for 3 days and for yeast and molds at 20-25°C for 5 days.
- After incubation, observe the plates and record the results in the validation report.

12.17. **Calculation:**

- Calculate the Log reduction as per the below formula:

  Log Reduction = Log 10 (Initial Count -Observed count)

13.0 Result Recording

Validation study performed in two parts and fallowing are the test performed during disinfectant validation and results recorded accordingly.

1.**Use dilution test:** To determine the effective concentration and to screen the use concentration at various contact times.

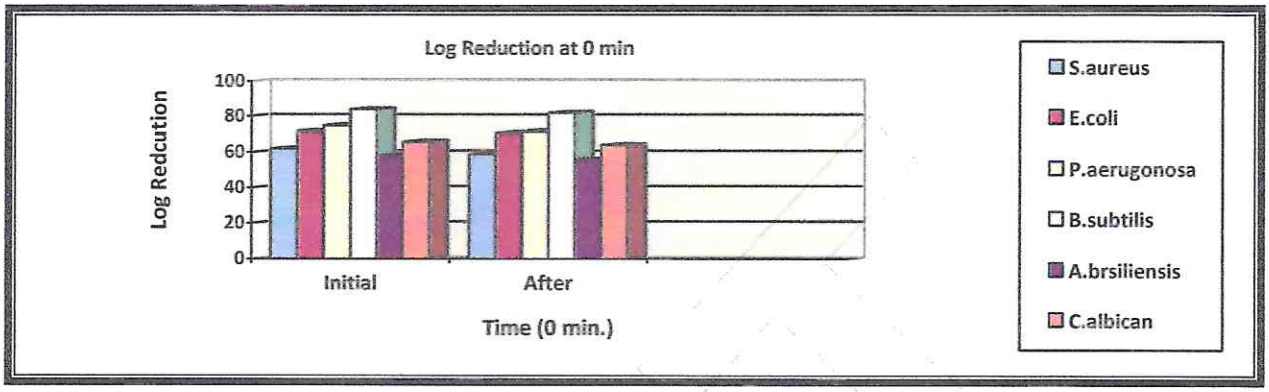
2. Surface challenge test or Surface recovery test: To determine the effectiveness of use concentration in reducing the bioburden on different surface.

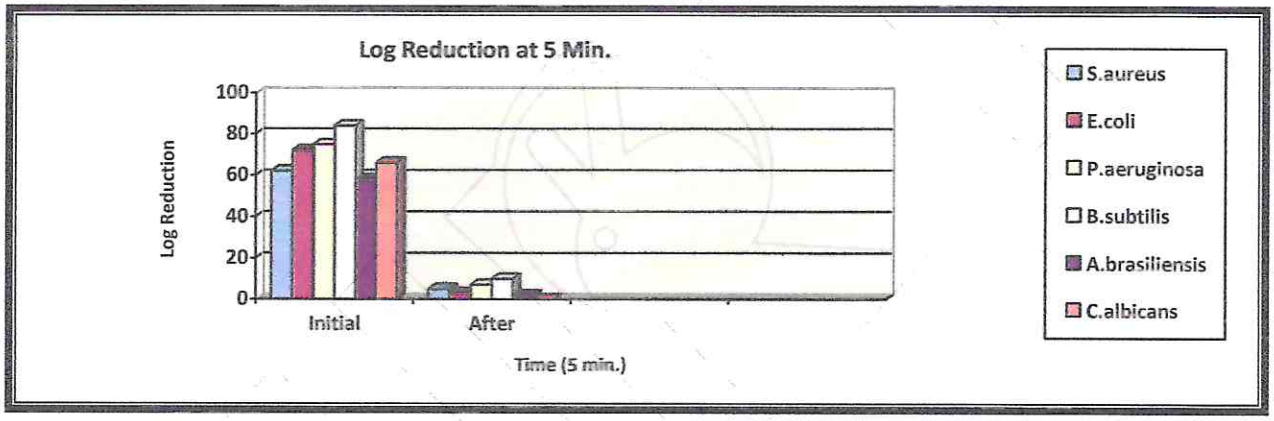
A. User Dilution test:

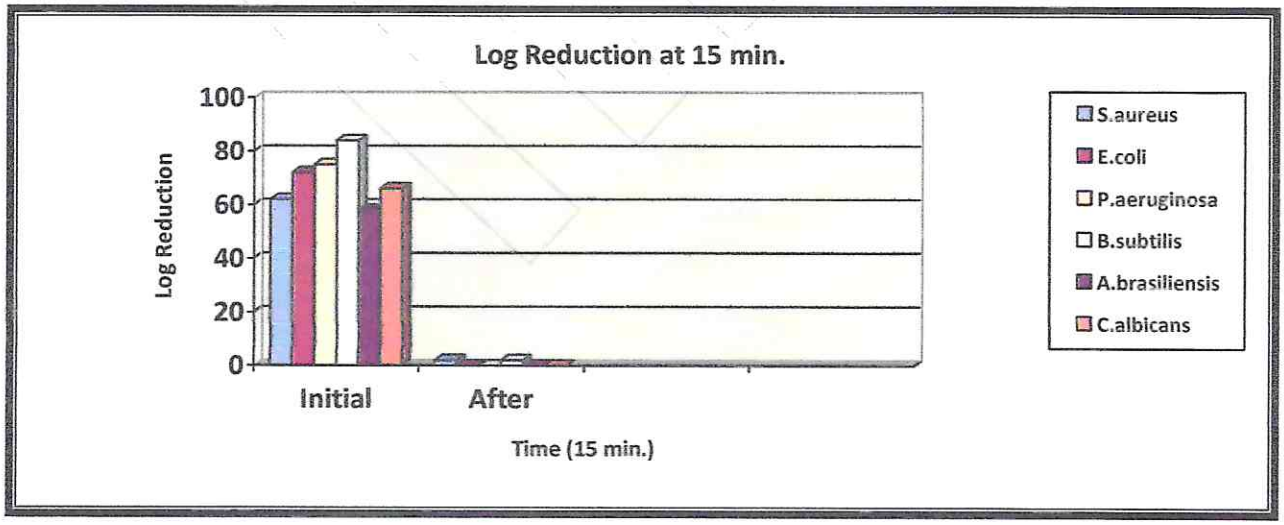
1. For disinfectant Virosil fallowing are the results:

Name	Virosil 5%	Date of Test:	23-01-2024
Media Used:	SCDA/SDA	Date of Result:	29-01-2024
Media Ref. No.:	SCDA230124 SDA230124	Incubator ID. (30-35C°):	MBD/INC-003
		Incubator ID. (20-25 C°):	MBD/INC-040
Incubation period (20-25 C°)	05 days	Incubation period (30-35C°)	05 days

Contact Time (In Min.)	Strains Used	Used Culture Suspension Concentration	Log	Observed cfu	Log	Log Reduction
0	S. aureus	62×10^5	6.792	59×10^5	6.771	0.021
	E.coli	72×10^5	6.857	71×10^5	6.851	0.006
	P. aeruginosa	75×10^5	6.875	72×10^5	6.857	0.018
	B.subtilis	84×10^5	6.924	82×10^5	6.914	0.010
	A.Brasiliensis	58×10^5	6.763	56×10^5	6.748	0.015
	C. albicans	66×10^5	6.820	64×10^5	6.806	0.014
5	S. aureus	62×10^5	6.792	5	0.699	6.093
	E.coli	72×10^5	6.857	3	0.447	6.410
	P. aeruginosa	75×10^5	6.875	7	0.845	6.030
	B.subtilis	84×10^5	6.924	10	1.000	5.924
	A.Brasiliensis	58×10^5	6.763	2	0.301	6.462
	C. albicans	66×10^5	6.820	00	--	6.820
15	S. aureus	62×10^5	6.792	02	0.301	6.491
	E.coli	72×10^5	6.857	00	--	6.857
	P. aeruginosa	75×10^5	6.875	00	--	6.875
	B.subtilis	84×10^5	6.924	02	0.301	6.623
	A.Brasiliensis	58×10^5	6.763	00	--	6.763
	C.albicans	66×10^5	6.820	00	--	6.820
Positive control	Growth Observed					
Negative control	No Growth					


Log Reduction at 0 min
Log Redcution
100
80
60
40
20
0
Initial
After
Time (0 min.)
S.aureus
E.coli
P.aerugonosa
B.subtilis
A.brsiliensis
C.albican


Log Reduction at 5 Min.
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5 min.)
S.aureus
E.coli
P.aeruginosa
B.subtilis
A.brasiliensis
C.albicans

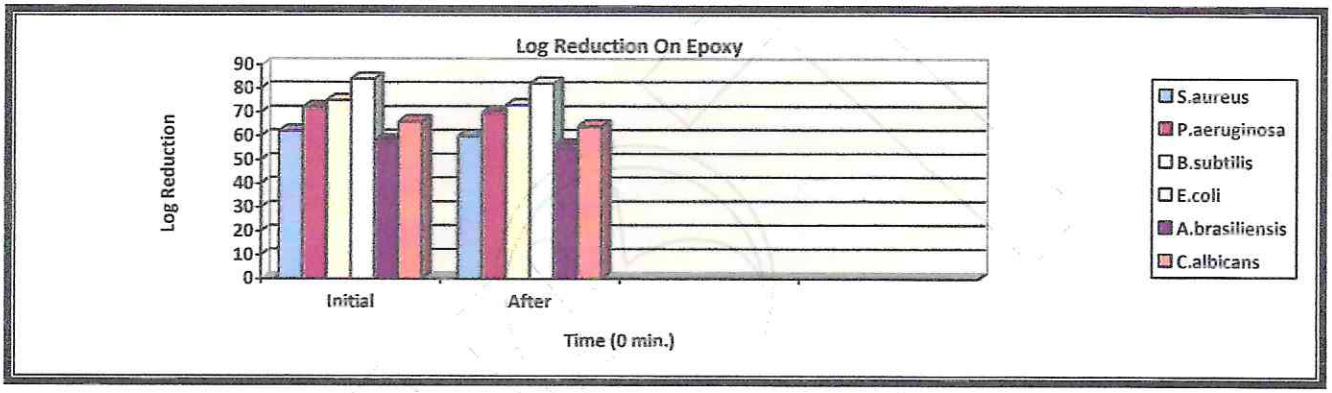

Log Reduction at 15 min.
Log Reduction
100
80
60
40
20
0
Initial
After
Time (15 min.)
S.aureus
E.coli
P.aeruginosa
B.subtilis
A.brasiliensis
C.albicans

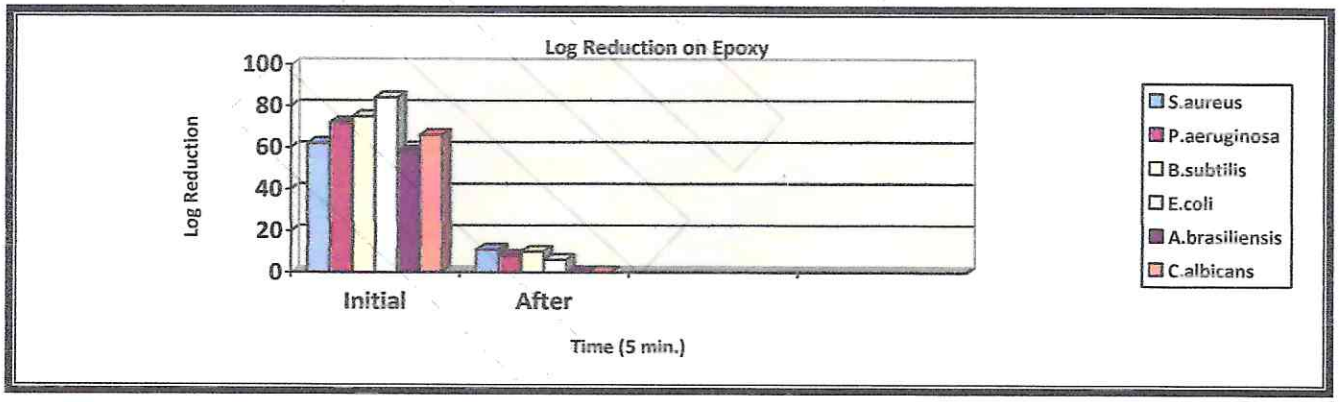
B. Surface Challenge/Recovery test Report: Fallowing are the results of each organism used against each surface with defined contact time and disinfectant concentration.

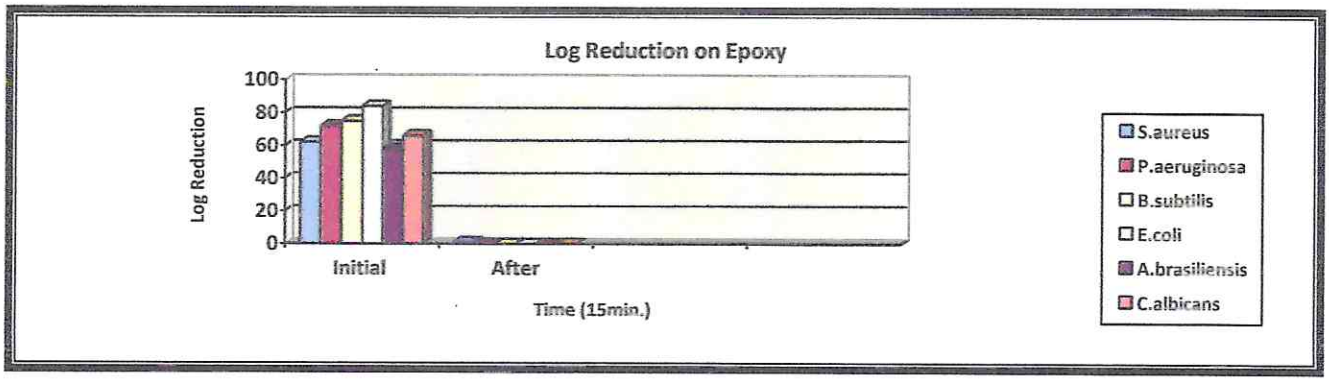
Contact surface:	Epoxy		
Date of Test:	24-01-2024	Date of Report:	29-01-2024
Disinfectant solution & concentration	Virosil (5%)	Media Ref. No.:	SCDA240124 SDA240124
Incubator ID:	MBD/INC-040 MBD/INC-003	Incubator temp./time:	20-25 C°/5days 30-35C°/5 days

Name of Culture	Initial counts	Log	Time (in min.)	Observed cfu after contact	Log	Log Reduction
S. aureus	62×10^5	6.792	0	60×10^5	6.778	0.377
P. aeruginosa	72×10^5	6.857		70×10^5	6.845	0.442
B.subtilis	75×10^5	6.875		73×10^5	6.863	0.295
E.coli	84×10^5	6.924		82×10^5	6.914	0.243
A.Brasiliensis	58×10^5	6.763		56×10^5	6.748	1.462
C. albicans	66×10^5	6.820		64×10^5	6.806	1.218
S. aureus	62×10^5	6.792	5	11	1.041	5.751
P. aeruginosa	72×10^5	6.857		08	0.903	5.954
B.subtilis	75×10^5	6.875		10	1.000	6.875
E.coli	84×10^5	6.924		06	0.778	6.146
A.Brasiliensis	58×10^5	6.763		00	--	6.763
C. albicans	66×10^5	6.820		00	--	6.820
S. aureus	62×10^5	6.792	15	01	--	6.792
P. aeruginosa	72×10^5	6.857		00	--	6.857
B.subtilis	75×10^5	6.875		00	--	6.875
E.coli	84×10^5	6.924		00	--	6.924

A.Brasiliensis	58×10^5	6.763		00	--	6.763
C. albicans	66×10^5	6.820		00	--	6.820
Positive control	Growth Observed					
Negative control	No Growth					



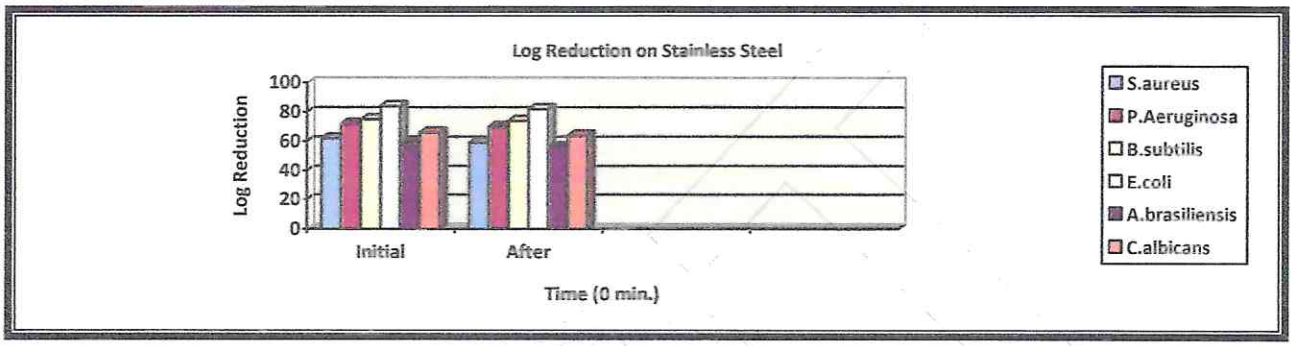


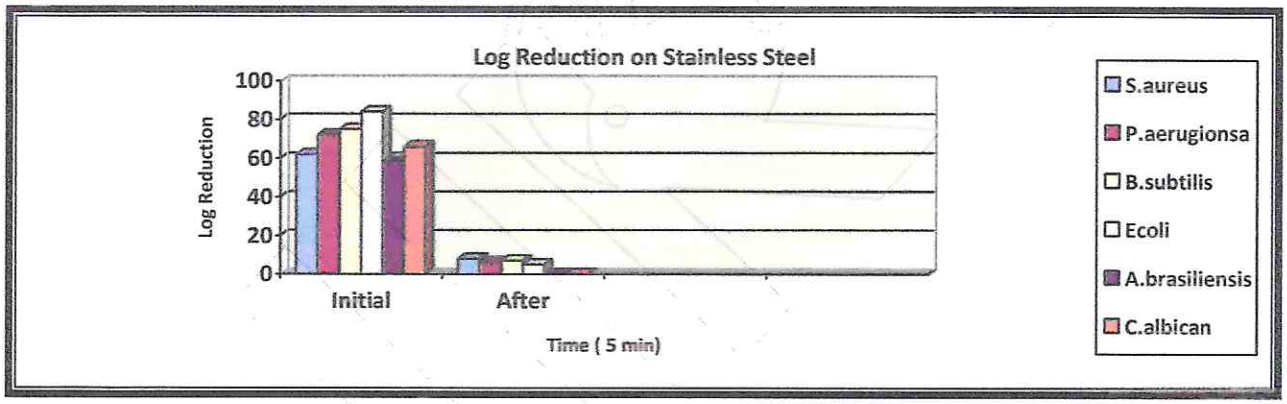


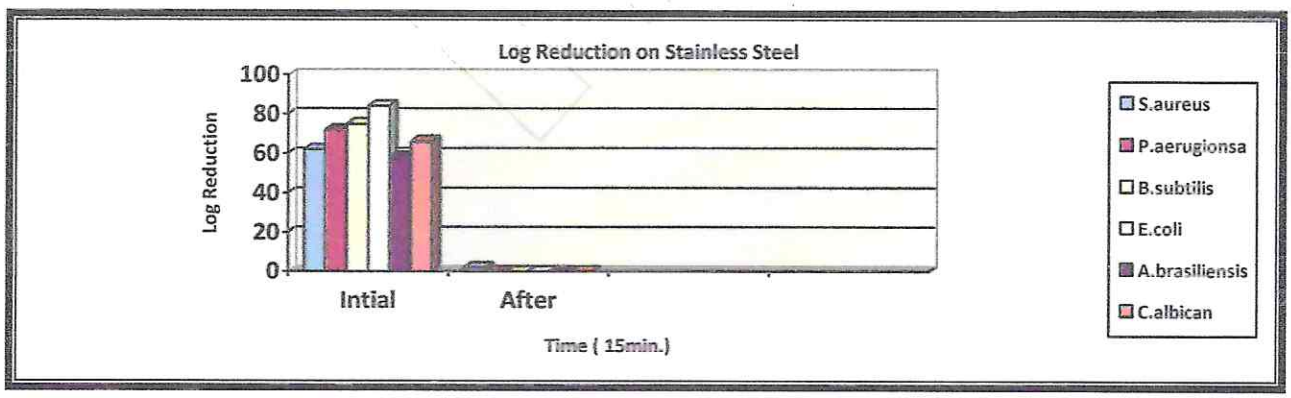
Contact surface:	Stainless Steel (316)		
Date of Test:	24-01-2024	Date of Report:	29-01-2024
Disinfectant solution & concentration	Virosil (5%)	Media Ref. No.:	SCDA240124 SDA240124
Incubator ID:	MBD/INC-040 MBD/INC-003	Incubator temp./time:	20-25 C°/5days 30-35C°/5 days

Name of Culture	Initial counts	Log	Time (in min.)	Observed cfu after contact	Log	Log Reduction
S. aureus	62×10^5	6.792	0	59×10^5	6.771	0.290
P. aeruginosa	72×10^5	6.857		70×10^5	6.845	0.426
B.subtilis	75×10^5	6.875		74×10^5	6.869	0.331
E.coli	84×10^5	6.924		82×10^5	6.914	0.208
A.Brasiliensis	58×10^5	6.763		56×10^5	6.748	0.683
C. albicans	66×10^5	6.820		64×10^5	6.806	0.343
S. aureus	62×10^5	6.792	5	8	0.903	6.069
P. aeruginosa	72×10^5	6.857		6	0.778	6.079
B.subtilis	75×10^5	6.875		7	0.845	6.030
E.coli	84×10^5	6.924		5	0.699	6.225
A.Brasiliensis	58×10^5	6.763		00	--	6.763
C. albicans	66×10^5	6.820		00	--	6.820
S. aureus	62×10^5	6.792	15	02	0.301	6.491
P. aeruginosa	72×10^5	6.857		00	--	6.857
B.subtilis	75×10^5	6.875		00	--	6.875
E.coli	84×10^5	6.924		00	--	6.924
A.Brasiliensis	66×10^5	6.820		00	--	6.820
C. albicans	62×10^5	6.792		00	--	6.792

Positive control	Growth Observed
Negative control	No Growth



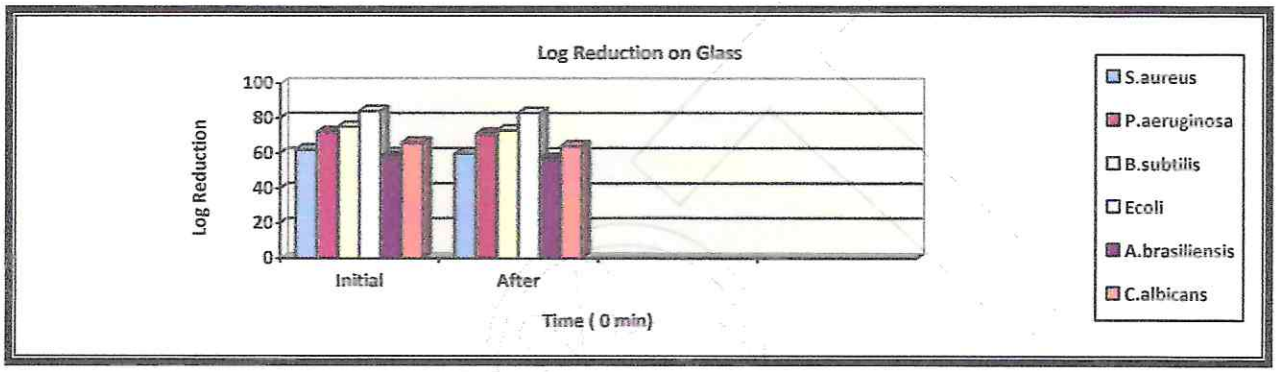


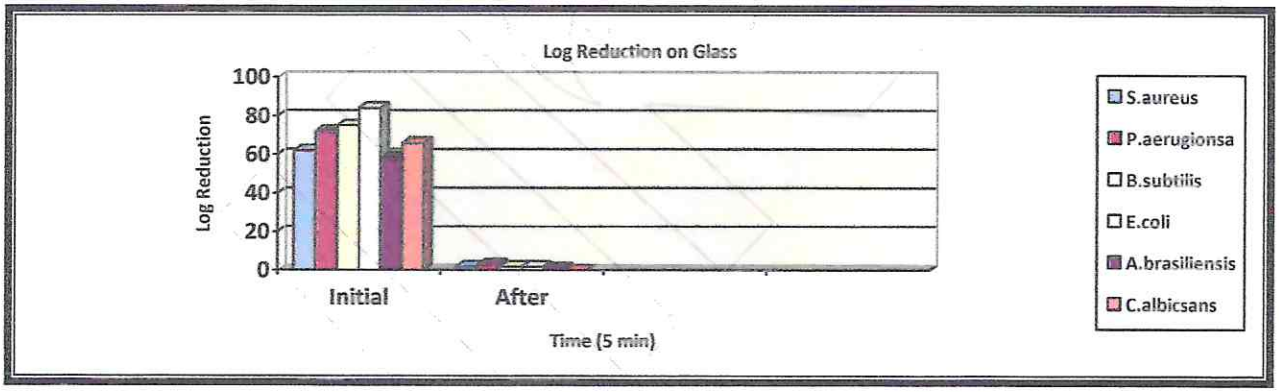


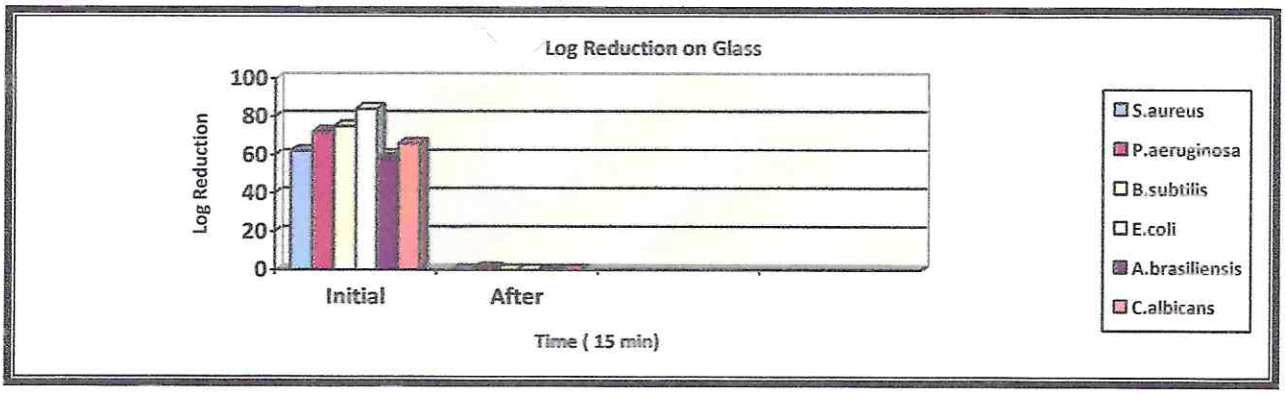
Contact surface:	Glass		
Date of Test:	24-01-2024	Date of Report:	29-01-2024
Disinfectant solution & concentration	Virosil (5%)	Media Ref. No.:	SCDA240124 SDA240124
Incubator ID:	MBD/INC-040 MBD/INC-003	Incubator temp./time:	20-25 C°/5days 30-35C°/5 days

Name of Culture	Initial counts	Log	Time (in min.)	Observed cfu after contact	Log	Log Reduction
S. aureus	62×10^5	6.792	0	60×10^5	6.778	0.014
P. aeruginosa	72×10^5	6.857		71×10^5	6.851	0.006
B.subtilis	75×10^5	6.875		73×10^5	6.863	0.012
E.coli	84×10^5	6.924		83×10^5	6.919	0.005
A.Brasiliensis	58×10^5	6.763		57×10^5	6.756	0.007
C. albicans	66×10^5	6.820		64×10^5	6.806	0.014
S. aureus	62×10^5	6.792	5	02	0.301	6.491
P. aeruginosa	72×10^5	6.857		03	0.477	6.380
B.subtilis	75×10^5	6.875		02	0.301	6.574
E.coli	84×10^5	6.924		02	0.301	6.623
A.Brasiliensis	58×10^5	6.763		01	--	6.763
C. albicans	66×10^5	6.820		00	--	6.820
S. aureus	62×10^5	6.792	15	00	--	6.792
P. aeruginosa	72×10^5	6.857		01	--	6.857
B.subtilis	75×10^5	6.875		00	--	6.875
E.coli	84×10^5	6.924		00	--	6.924
A.Brasiliensis	58×10^5	6.792		00	--	6.792

C. albicans	66 $\times 10^5$	6.820		00	--	6.820
Positive control	Growth Observed					
Negative control	No Growth					



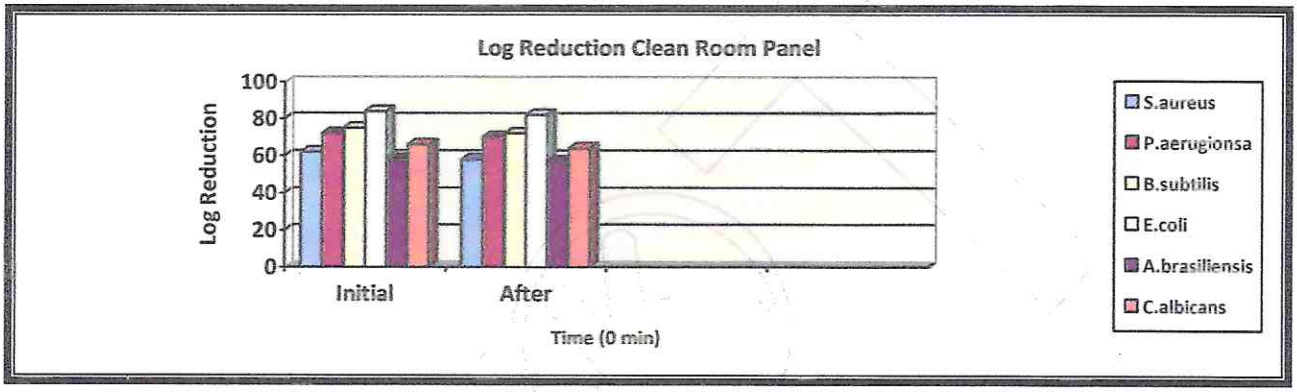


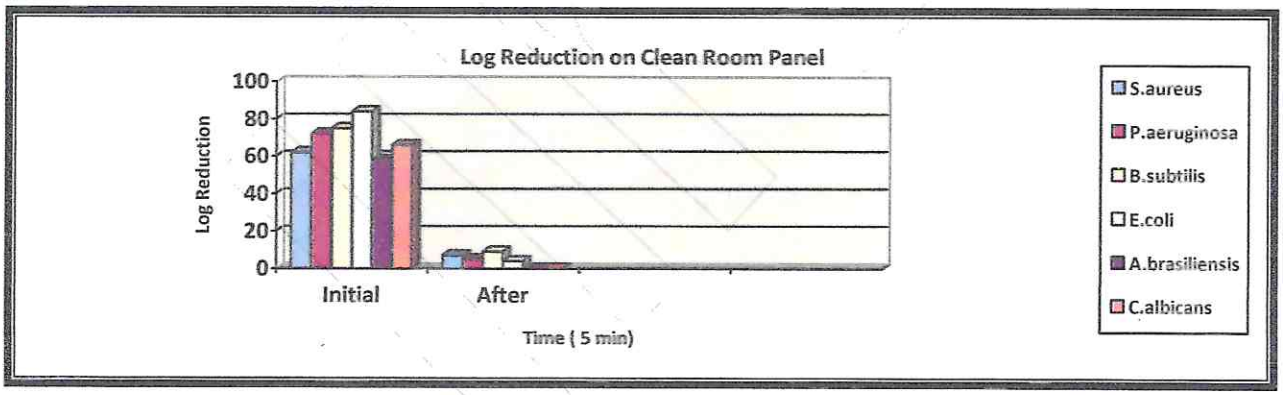


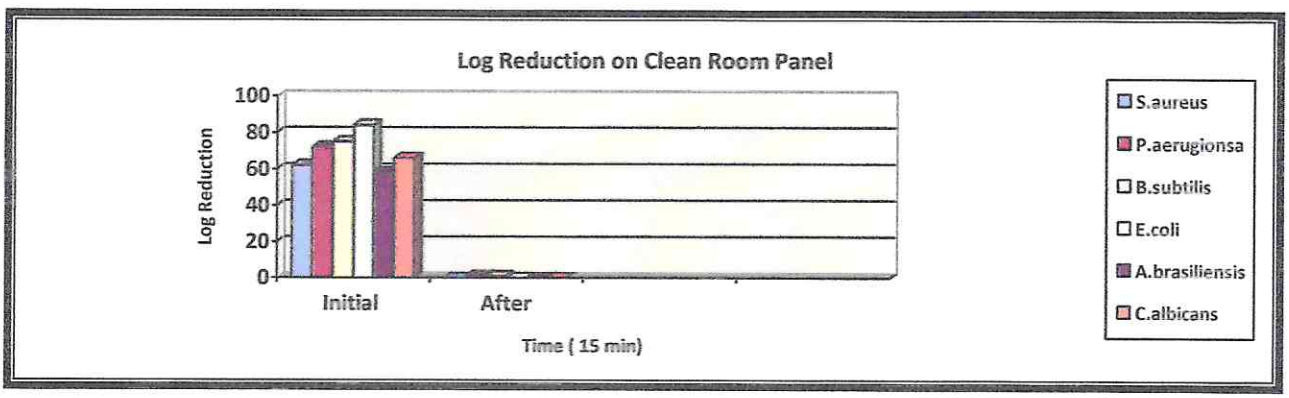
Contact surface:	Clean Room Panel		
Date of Test:	24-01-2024	Date of Report:	29-01-2024
Disinfectant solution & concentration	Virosil (5%)	Media Ref. No.:	SCDA240124 SDA240124
Incubator ID:	MBD/INC-040 MBD/INC-003	Incubator temp./time:	20-25 C°/5days 30-35C°/5 days

Name of Culture	Initial counts	Log	Time (in min.)	Observed cfu after contact	Log	Log Reduction
S. aureus	62×10^5	6.792	0	58×10^5	6.763	0.029
P. aeruginosa	72×10^5	6.857		70×10^5	6.845	0.012
B.subtilis	75×10^5	6.875		72×10^5	6.857	0.018
E.coli	84×10^5	6.924		82×10^5	6.914	0.010
A.Brasiliensis	58×10^5	6.763		57×10^5	6.756	0.007
C. albicans	66×10^5	6.820		64×10^5	6.806	0.014
S. aureus	62×10^5	6.792	5	07	0.845	5.947
P. aeruginosa	72×10^5	6.857		05	0.699	6.158
B.subtilis	75×10^5	6.875		09	0.954	5.921
E.coli	84×10^5	6.924		04	0.602	6.322
A.Brasiliensis	58×10^5	6.763		00	--	6.763
C. albicans	66×10^5	6.820		00	--	6.820
S. aureus	62×10^5	6.792	15	00	--	6.792
P. aeruginosa	72×10^5	6.857		01	--	6.857
B.subtilis	75×10^5	6.875		01	--	6.875
E.coli	84×10^5	6.924		00	--	6.924
A.Brasiliensis	58×10^5	6.792		00	--	6.792

C. albicans	66×10^5	6.820		00	--	6.820
Positive control	Growth Observed					
Negative control	No Growth					



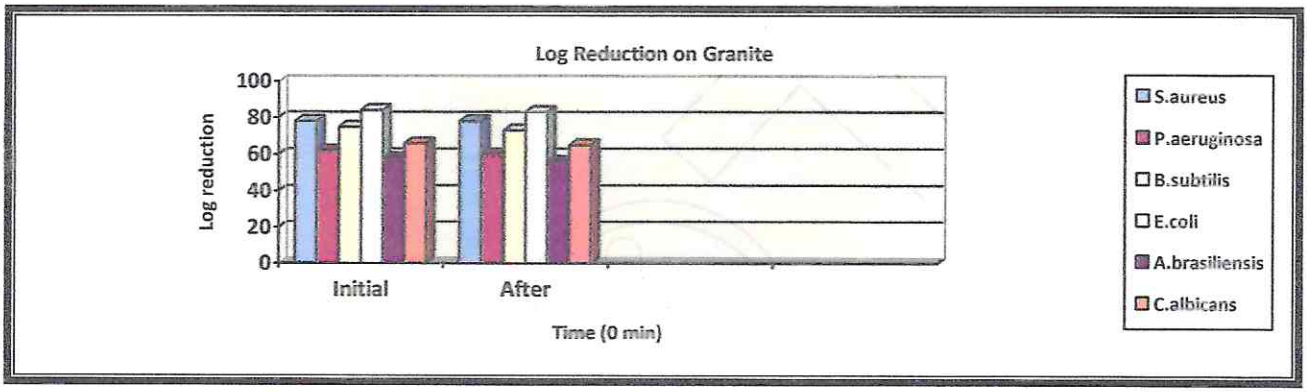


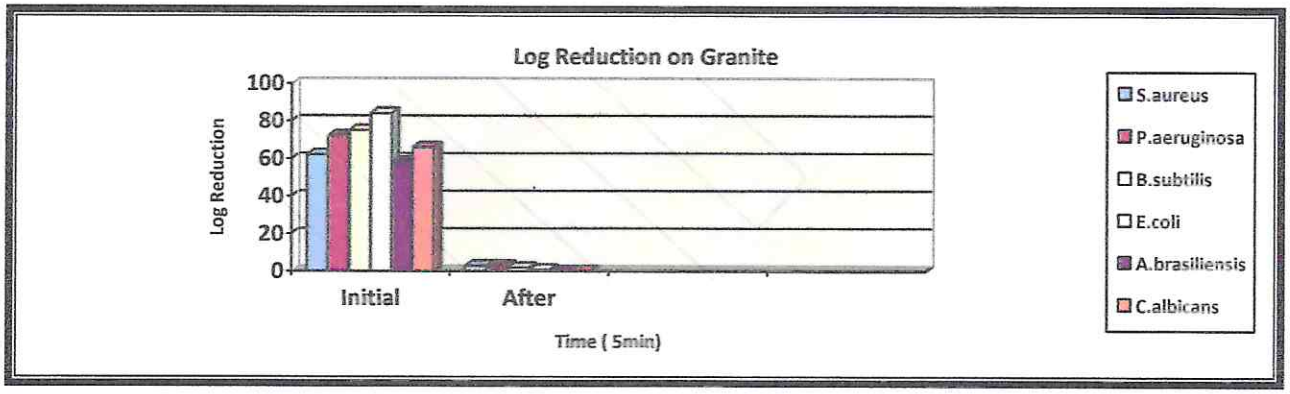


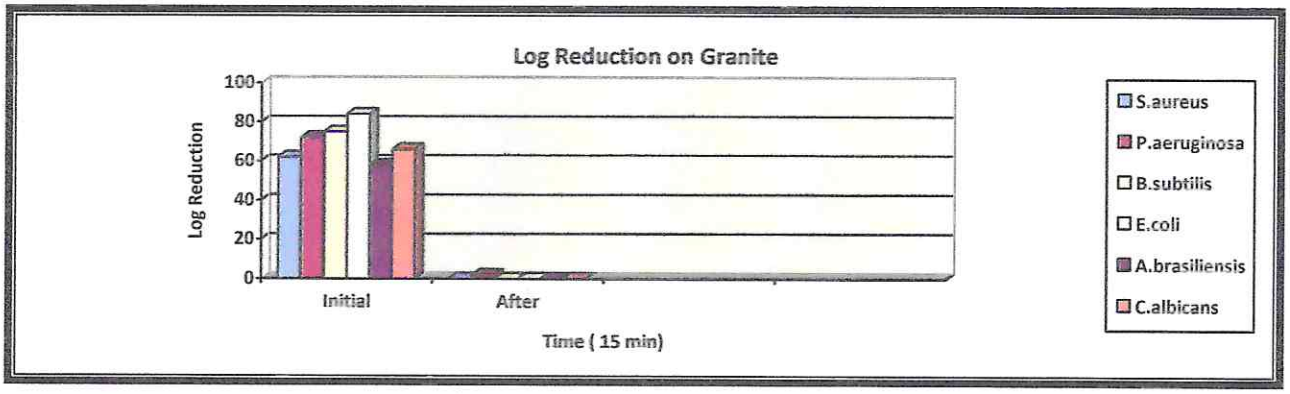
Contact surface:	Granite		
Date of Test:	24-01-2024	Date of Report:	29-01-2024
Disinfectant solution & concentration	Virosil (5%)	Media Ref. No.:	SCDA240124 SDA240124
Incubator ID:	MBD/INC-040 MBD/INC-003	Incubator temp./time:	20-25 C°/5days 30-35C°/5 days

Name of Culture	Initial counts	Log	Time (in min.)	Observed cfu after contact	Log	Log Reduction
S. aureus	62×10^5	6.792	0	60×10^3	6.778	0.014
P. aeruginosa	72×10^5	6.857		69×10^3	6.839	0.018
B.subtilis	75×10^5	6.875		73×10^3	6.863	0.012
E.coli	84×10^5	6.924		83×10^3	6.919	0.005
A.Brasiliensis	58×10^5	6.763		56×10^3	6.748	0.015
C. albicans	66×10^5	6.820		65×10^3	6.813	0.007
S. aureus	62×10^5	6.792	5	3	0.477	6.315
P. aeruginosa	72×10^5	6.857		3	0.477	6.38
B.subtilis	75×10^5	6.875		2	0.301	6.574
E.coli	84×10^5	6.924		1	--	6.924
A.Brasiliensis	58×10^5	6.763		00	--	6.763
C. albicans	66×10^5	6.820		00	--	6.820
S. aureus	62×10^5	6.792	15	00	--	6.792
P. aeruginosa	72×10^5	6.857		01	--	6.857
B.subtilis	75×10^5	6.875		00	--	6.875
E.coli	84×10^5	6.924		00	--	6.924
A.Brasiliensis	58×10^5	6.792		00	--	6.792

C. albicans	66×10^5	6.820		00	--	6.820
Positive control	Growth Observed					
Negative control	No Growth					







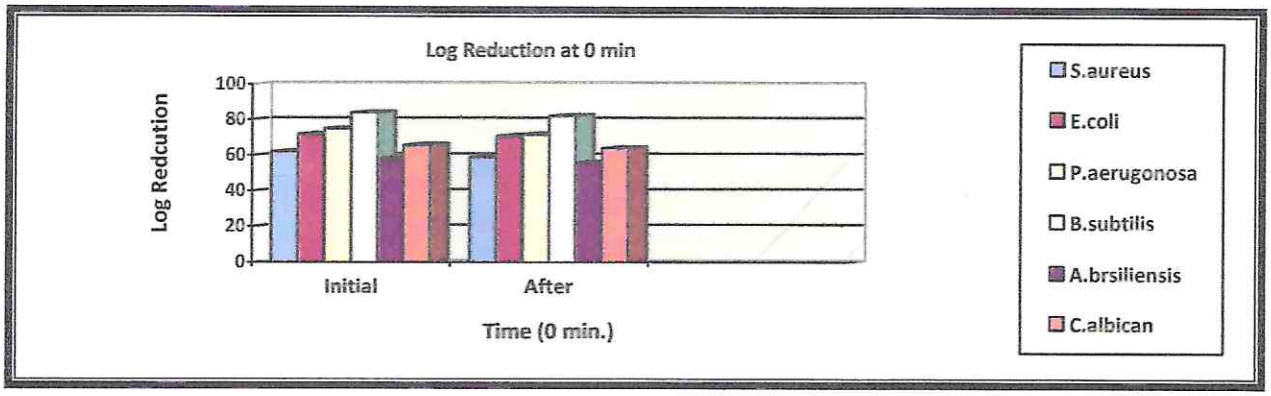
14.0 CONCLUSION:

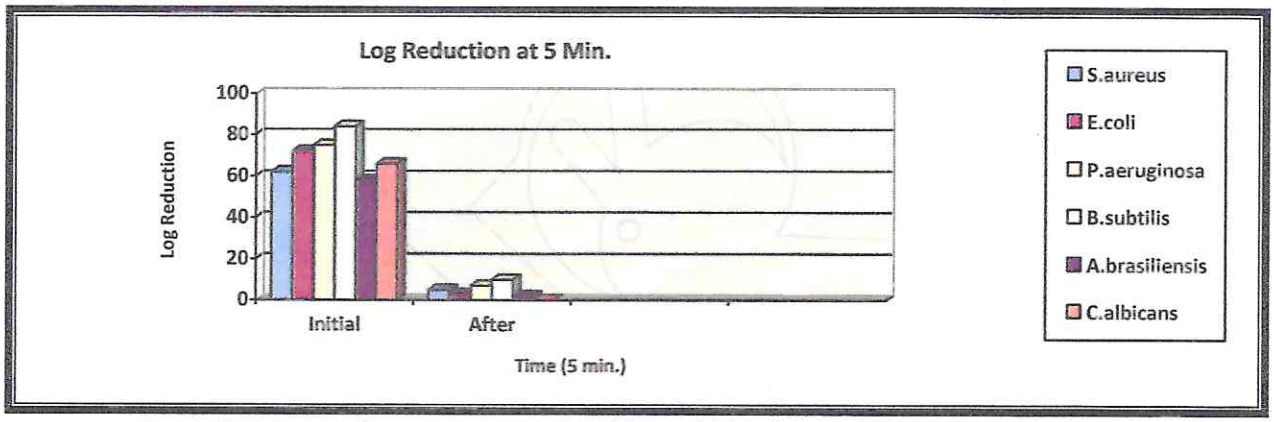
Following observation are found during disinfectant validation study performed as per protocol "Disinfectant Validation of Virosil" protocol no. IIRT/VER/MBD/24-P01.

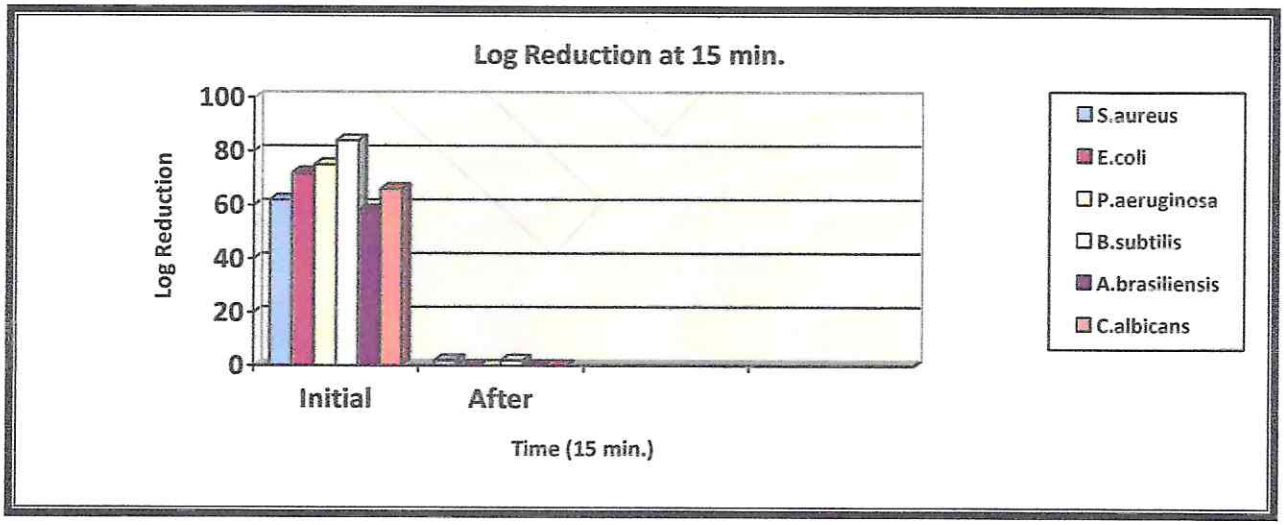
Name of Test	**Dilution Method**	
Name of Microorganism	ATCC/NCIM no.	Log Reduction (0 min.)
S. aureus	6538P/2079	0.021
P. aeruginosa	9027/2200	0.006
B.subtilis	6633/2063	0.018
E.coli	8739/2065	0.010
A.Brasiliensis	16404/1196	0.015
C. albicans	10231/3471	0.014

Name of Test	**Dilution Method**	
Name of Microorganism	ATCC/ NCIM no.	Log Reduction (5 min.)
S. aureus	6538P/2079	6.093
P. aeruginosa	9027/2200	6.410
B.subtilis	6633/2063	6.030
E.coli	8739/2065	5.924
A.Brasiliensis	16404/1196	6.462
C. albicans	10231/3471	6.820

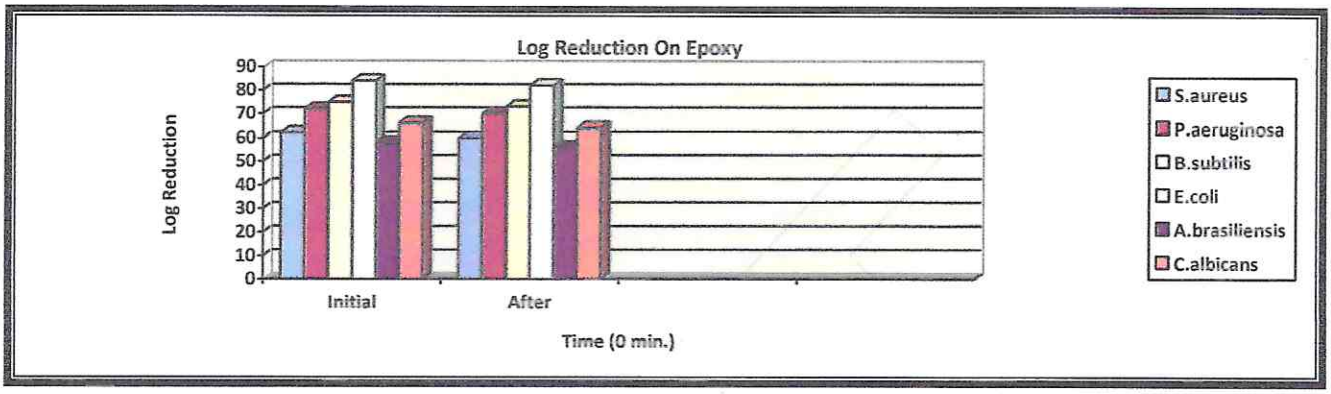
Name of Test	**Dilution Method**	
Name of Microorganism	ATCC/ NCIM no.	Log Reduction (15 min.)
S. aureus	6538P/2079	6.491
P. aeruginosa	9027/2200	6.857
B.subtilis	6633/2063	6.875
E.coli	8739/2065	6.623
A.Brasiliensis	16404/1196	6.763
C. albicans	10231/3471	6.820

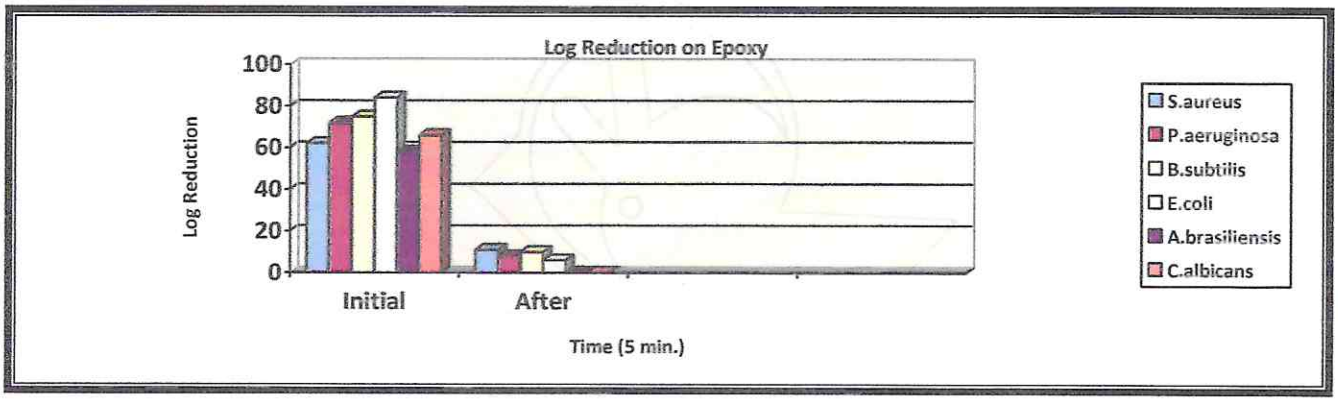

Log Reduction at 0 min
Log Redcution
100
80
60
40
20
0
Initial
After
Time (0 min.)
S.aureus
E.coli
P.aerugonosa
B.subtilis
A.brsiliensis
C.albican

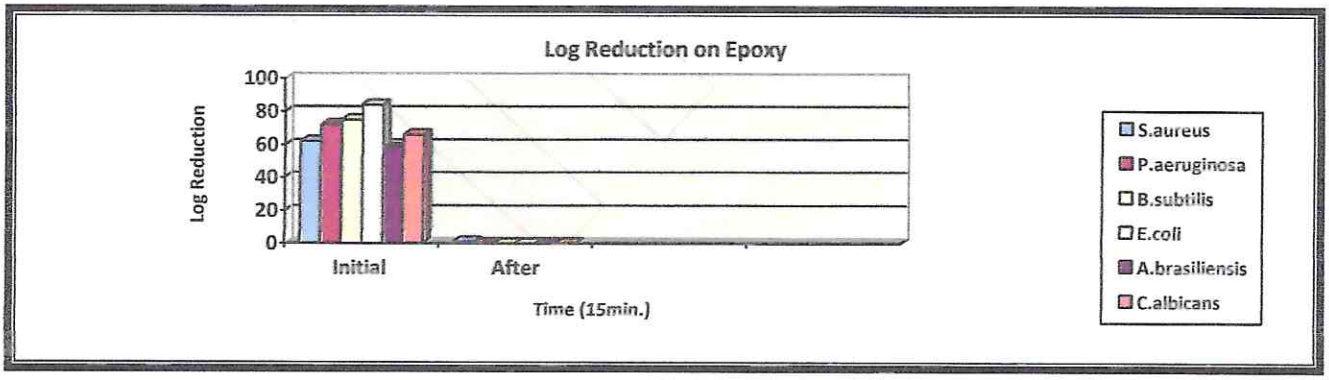

Log Reduction at 5 Min.
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5 min.)
S.aureus
E.coli
P.aeruginosa
B.subtilis
A.brasiliensis
C.albicans


Log Reduction at 15 min.
Log Reduction
100
80
60
40
20
0
Initial
After
Time (15 min.)
S.aureus
E.coli
P.aeruginosa
B.subtilis
A.brasiliensis
C.albicans

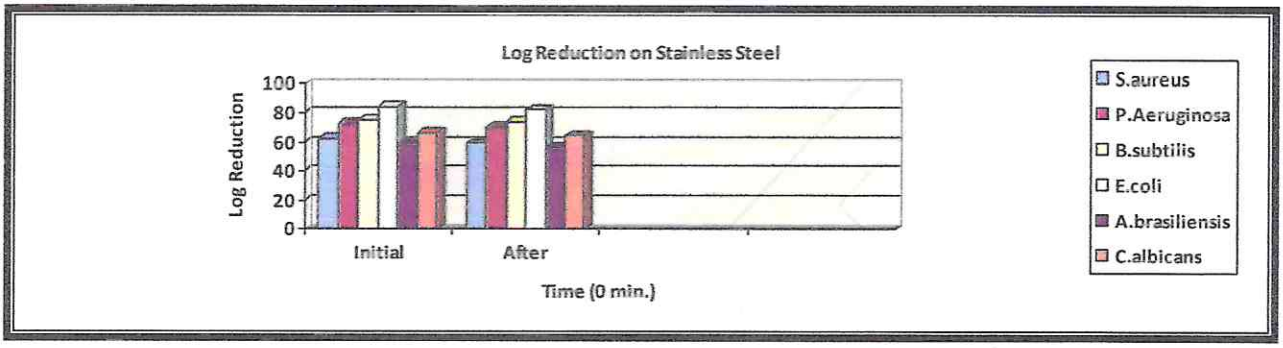
Name of Test	Surface Swab Method (Epoxy)		
	Name of Microorganism	ATCC/NCIM no.	Log Reduction
Epoxy (0 min.)	S. aureus	6538P/2079	0.377
	P. aeruginosa	9027/2200	0.442
	B.subtilis	6633/2063	0.295
	E.coli	8739/2065	0.243
	A.Brasiliensis	16404/1196	1.462
	C. albicans	10231/3471	1.218
Epoxy (5 min.)	S. aureus	6538P/2079	5.751
	P. aeruginosa	9027/2200	5.954
	B.subtilis	6633/2063	6.875
	E.coli	8739/2065	6.146
	A.Brasiliensis	16404/1196	6.763
	C. albicans	10231/3471	6.820
Epoxy (15 min.)	S. aureus	6538P/2079	6.792
	P. aeruginosa	9027/2200	6.857
	B.subtilis	6633/2063	6.875
	E.coli	8739/2065	6.924
	A.Brasiliensis	16404/1196	6.763
	C. albicans	10231/3471	6.820

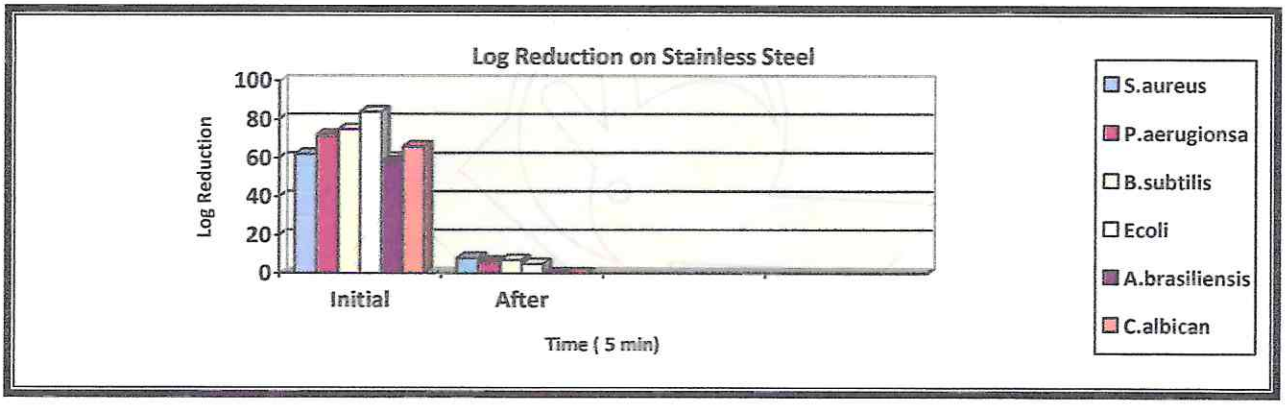

Log Reduction On Epoxy
Log Reduction
90
80
70
60
50
40
30
20
10
0
Initial
After
Time (0 min.)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans

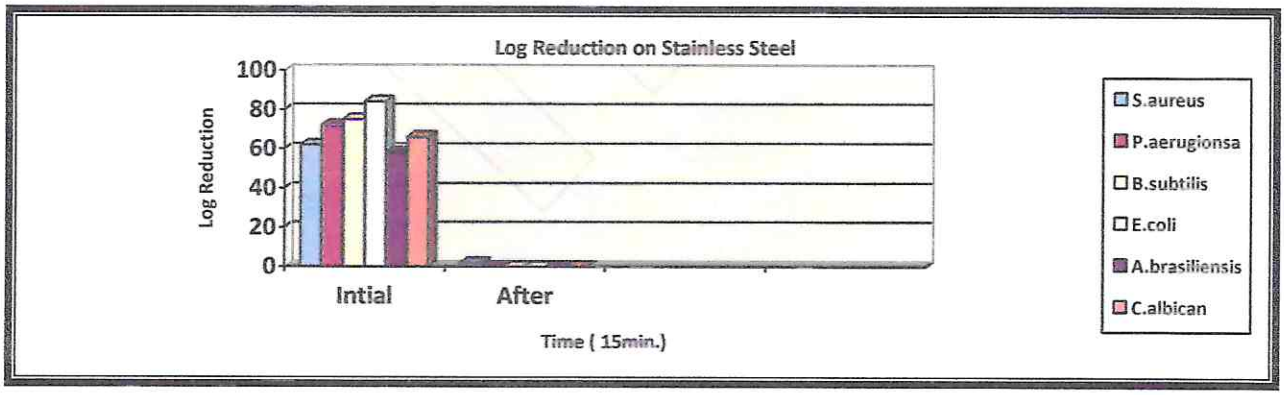

Log Reduction on Epoxy
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5 min.)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans


Log Reduction on Epoxy
Log Reduction
100
80
60
40
20
0
Initial
After
Time (15min.)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans

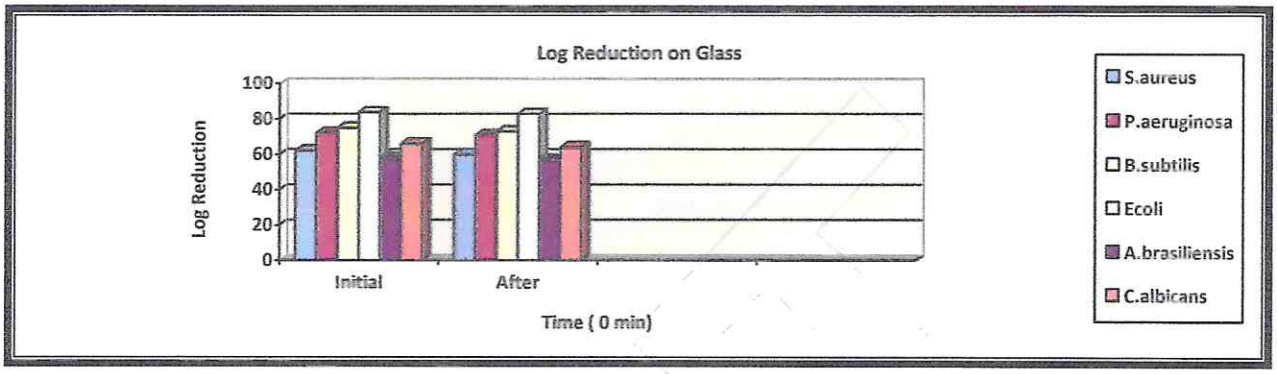
Name of Test	Surface Swab Method (Stainless Steel 316)		
	Name of Microorganism	ATCC/NCIM no.	Log Reduction
Stainless Steel (0 min.)	S. aureus	6538P/2079	0.290
	P. aeruginosa	9027/2200	0.426
	B.subtilis	6633/2063	0.331
	E.coli	8739/2065	0.208
	A.Brasiliensis	16404/1196	0.683
	C. albicans	10231/3471	0.343
Stainless Steel (5 min.)	S. aureus	6538P/2079	6.069
	P. aeruginosa	9027/2200	6.079
	B.subtilis	6633/2063	6.030
	E.coli	8739/2065	6.225
	A.Brasiliensis	16404/1196	6.763
	C. albicans	10231/3471	6.820
Stainless Steel (15 min.)	S. aureus	6538P/2079	6.491
	P. aeruginosa	9027/2200	6.857
	B.subtilis	6633/2063	6.875
	E.coli	8739/2065	6.924
	A.Brasiliensis	16404/1196	6.820
	C. albicans	10231/3471	6.792

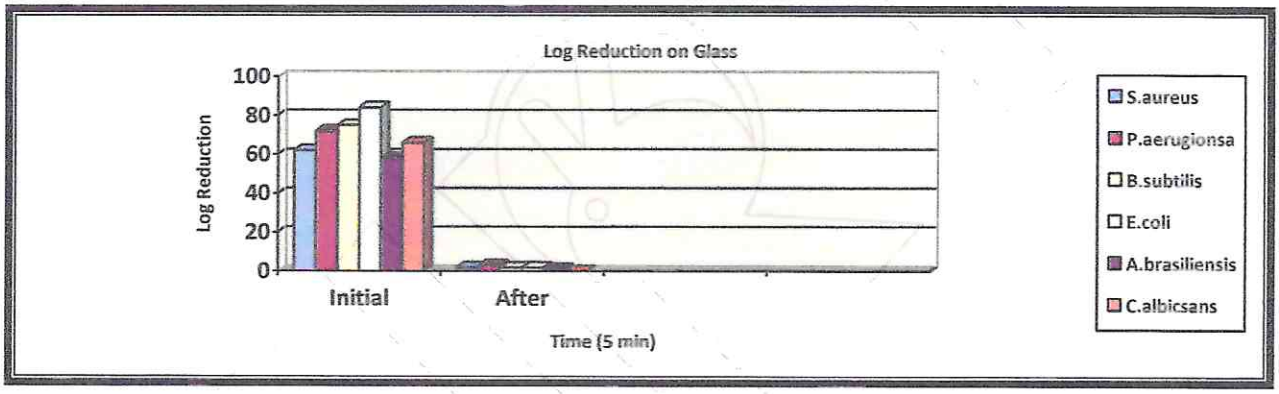

Log Reduction on Stainless Steel
Log Reduction
100
80
60
40
20
0
Initial
After
Time (0 min.)
S.aureus
P.Aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans

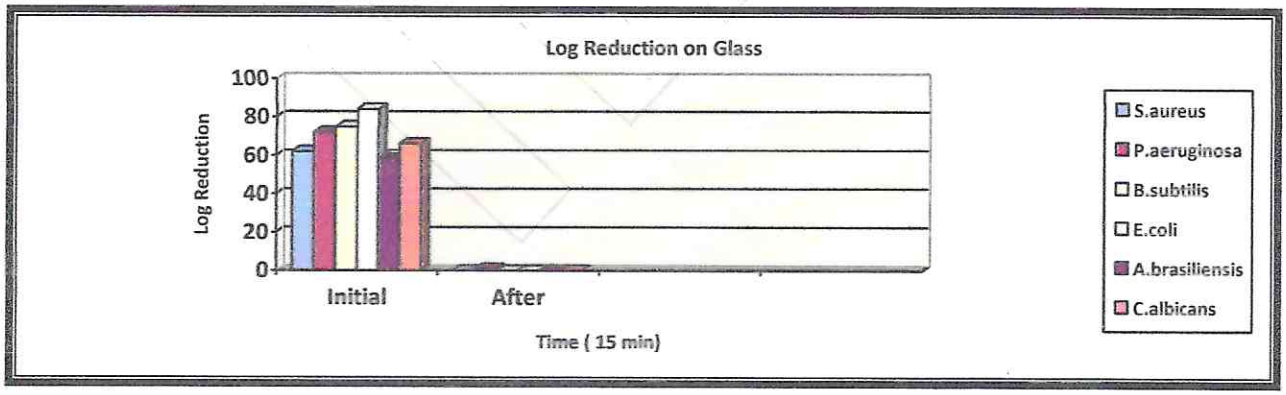

Log Reduction on Stainless Steel
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5 min)
S.aureus
P.aerugionsa
B.subtilis
Ecoli
A.brasiliensis
C.albican


Log Reduction on Stainless Steel
Log Reduction
100
80
60
40
20
0
Intial
After
Time (15min.)
S.aureus
P.aerugionsa
B.subtilis
E.coli
A.brasiliensis
C.albican

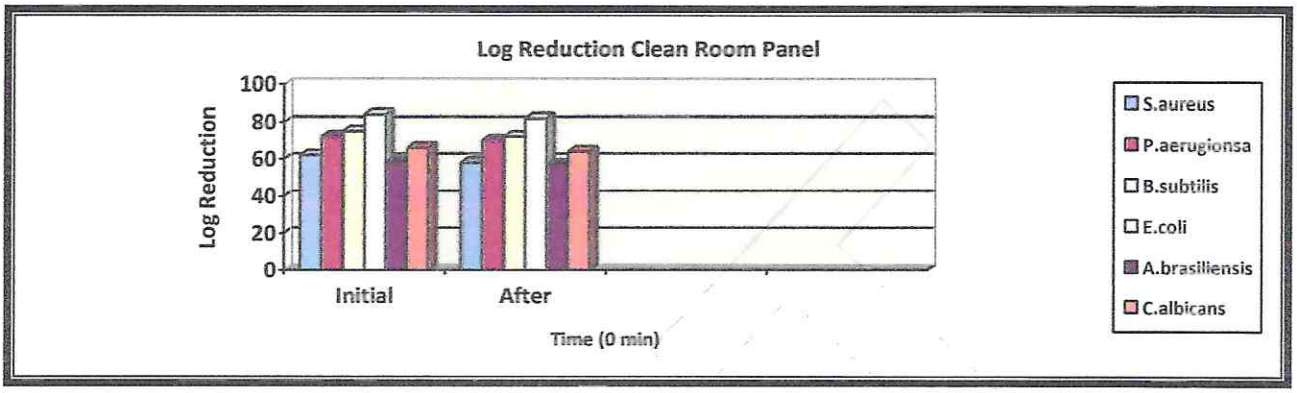
Name of Test	**Surface Swab Method (Glass)**		
	Name of Microorganism	ATCC/NCIM no.	Log Reduction
Glass (0 min.)	S. aureus	6538P/2079	0.014
	P. aeruginosa	9027/2200	0.006
	B.subtilis	6633/2063	0.012
	E.coli	8739/2065	0.005
	A.Brasiliensis	16404/1196	0.007
	C. albicans	10231/3471	0.014
Glass (5 min.)	S. aureus	6538P/2079	6.491
	P. aeruginosa	9027/2200	6.380
	B.subtilis	6633/2063	6.574
	E.coli	8739/2065	6.623
	A.Brasiliensis	16404/1196	6.763
	C. albicans	10231/3471	6.820
Glass (15 min.)	S. aureus	6538P/2079	6.792
	P. aeruginosa	9027/2200	6.857
	B.subtilis	6633/2063	6.875
	E.coli	8739/2065	6.924
	A.Brasiliensis	16404/1196	6.792
	C. albicans	10231/3471	6.820

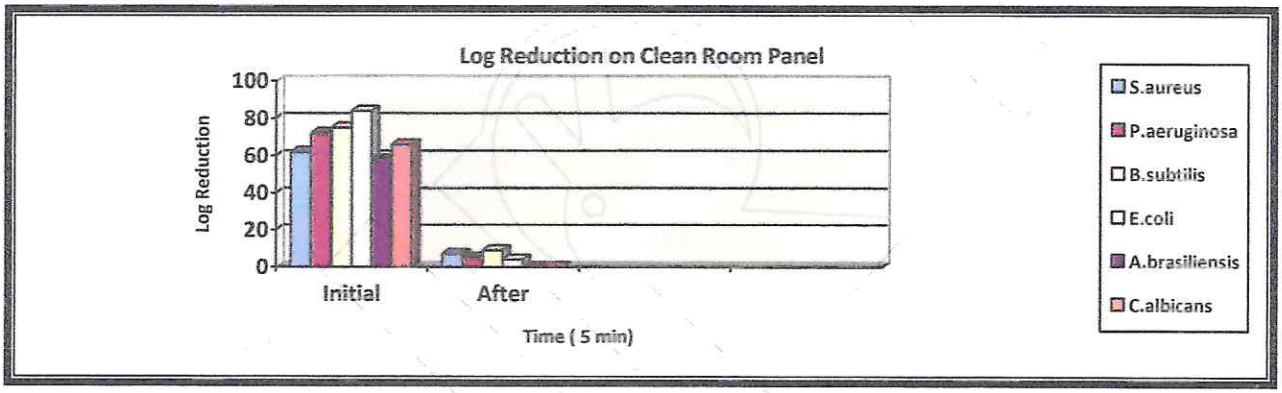

Log Reduction on Glass
Log Reduction
100
80
60
40
20
0
Initial
After
Time (0 min)
S.aureus
P.aeruginosa
B.subtilis
Ecoli
A.brasiliensis
C.albicans

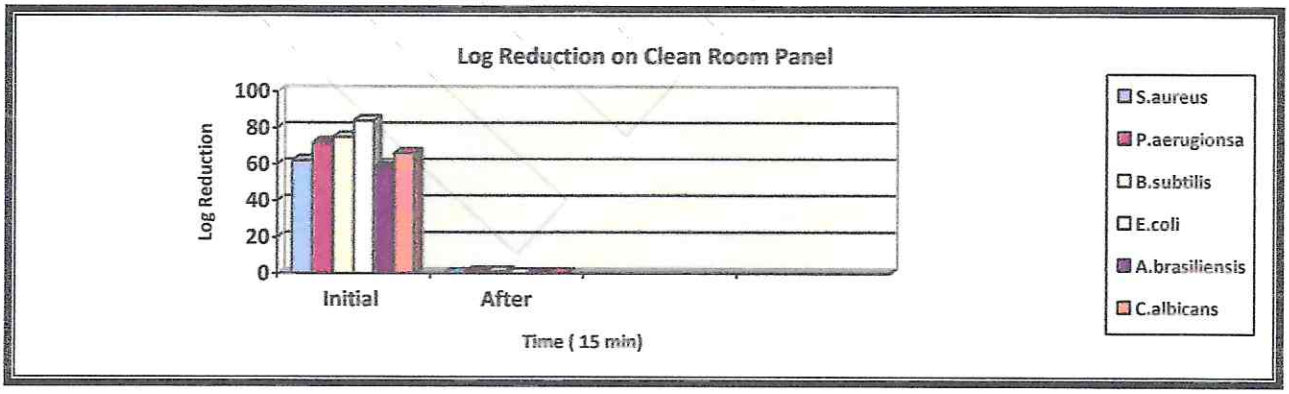

Log Reduction on Glass
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5 min)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans


Log Reduction on Glass
Log Reduction
100
80
60
40
20
0
Initial
After
Time (15 min)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans

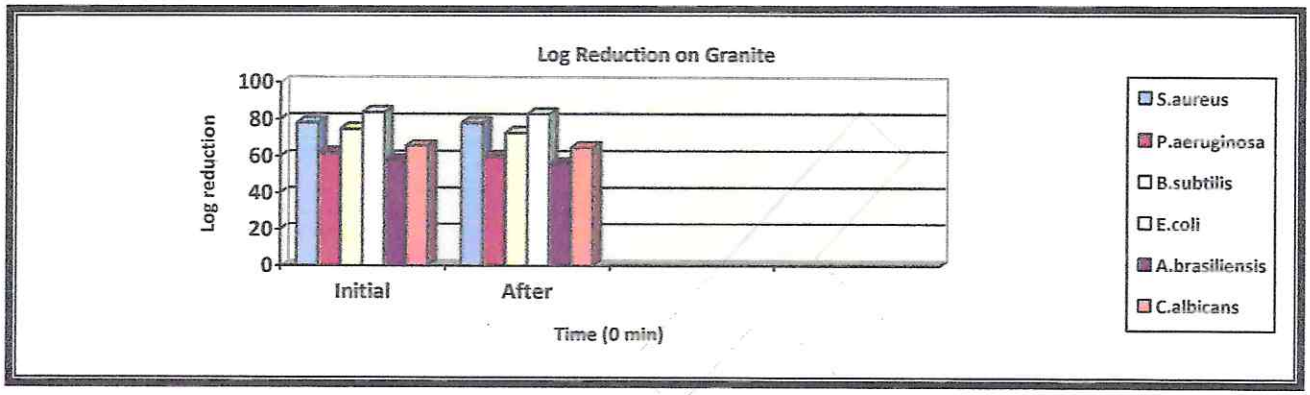
Name of Test	**Surface Swab Method (Clean Room Panel)**		
	Name of Microorganism	ATCC/NCIM no.	Log Reduction
Clean Room Panel (0 min.)	S. aureus	6538P/2079	0.029
	P. aeruginosa	9027/2200	0.012
	B.subtilis	6633/2063	0.018
	E.coli	8739/2065	0.010
	A.Brasiliensis	16404/1196	0.007
	C. albicans	10231/3471	0.014
Clean Room Panel (5 min.)	S. aureus	6538P/2079	5.947
	P. aeruginosa	9027/2200	6.158
	B.subtilis	6633/2063	5.921
	E.coli	8739/2065	6.322
	A.Brasiliensis	16404/1196	6.763
	C. albicans	10231/3471	6.820
Clean Room Panel (15 min.)	S. aureus	6538P/2079	6.792
	P. aeruginosa	9027/2200	6.857
	B.subtilis	6633/2063	6.875
	E.coli	8739/2065	6.924
	A.Brasiliensis	16404/1196	6.792
	C. albicans	10231/3471	6.820

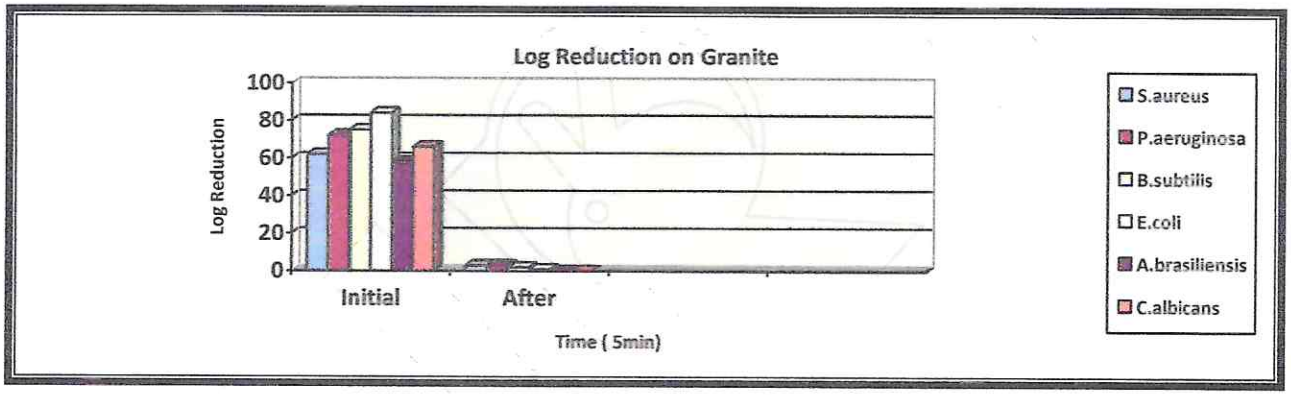

Log Reduction Clean Room Panel
Log Reduction
100
80
60
40
20
0
Initial
After
Time (0 min)
S.aureus
P.aerugionsa
B.subtilis
E.coli
A.brasiliensis
C.albicans

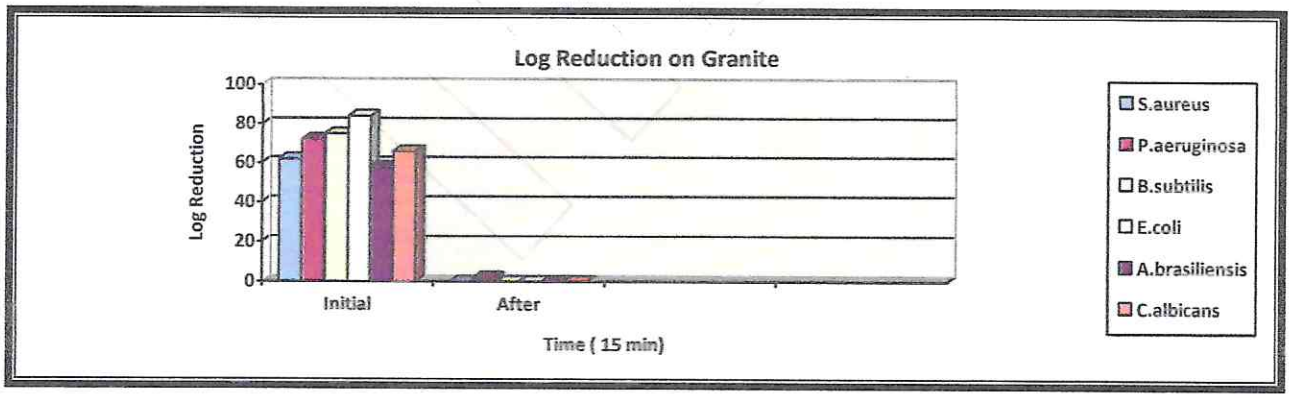

Log Reduction on Clean Room Panel
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5 min)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans


Log Reduction on Clean Room Panel
Log Reduction
100
80
60
40
20
0
Initial
After
Time (15 min)
S.aureus
P.aerugionsa
B.subtilis
E.coli
A.brasiliensis
C.albicans

Nampe of Test	**Surface Swab Method (Granite)**		
	Name of Microorganism	ATCC/NCIM no.	Log Reduction
Granite (0 min.)	S. aureus	6538P/2079	0.014
	P. aeruginosa	9027/2200	0.018
	B.subtilis	6633/2063	0.012
	E.coli	8739/2065	0.005
	A.Brasiliensis	16404/1196	0.015
	C. albicans	10231/3471	0.007
Granite (5 min.)	S. aureus	6538P/2079	6.315
	P. aeruginosa	9027/2200	6.38
	B.subtilis	6633/2063	6.574
	E.coli	8739/2065	6.924
	A.Brasiliensis	16404/1196	6.763
	C. albicans	10231/3471	6.820
Granite (15 min.)	S. aureus	6538P/2079	6.792
	P. aeruginosa	9027/2200	6.857
	B.subtilis	6633/2063	6.875
	E.coli	8739/2065	6.924
	A.Brasiliensis	16404/1196	6.792
	C. albicans	10231/3471	6.820


Log Reduction on Granite
Log reduction
100
80
60
40
20
0
Initial
After
Time (0 min)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans


Log Reduction on Granite
Log Reduction
100
80
60
40
20
0
Initial
After
Time (5min)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans


Log Reduction on Granite
Log Reduction
100
80
60
40
20
0
Initial
After
Time (15 min)
S.aureus
P.aeruginosa
B.subtilis
E.coli
A.brasiliensis
C.albicans

15.0 ACCEPTANCE CRITERIA:

- Disinfectant should achieve at least 3 log reduction for vegetative microorganisms and at least 2 log reduction for spore forming microorganisms.
- No growth should be observed in media negative control and process negative control.

16.0 INCIDENT/DEVIATION:

No any incident or deviation happened during this validation activity.

17.0 SUMMARY:

Following observation found during disinfectant validation study

- Total six microorganism (Spore forming and non-spore forming) used during validation study against one disinfectant.
- One concentration of disinfectant (the concentration recommended by manufacturer) with different contact time are used against known concentration of microorganism.
- Different surfaces are used in this validation study and found that Virosil 5 % v/v are effective against known microorganism on all available surfaces (i.e Epoxy, Glass, Stainless steel, Clean Room panel & Granite).
- As per results obtained it was observed that the Virosil 5% gives the minimum 6 log reduction for Spore forming and Non-spore forming microorganism within a contact time of 5 min. & 15 min. on all the microorganism as recommended by USP in both test i.e. Dilution method and Surface swab method.
- Hence it was concluded that the results of Virosil 5% are satisfactory as per the acceptance criteria i.e disinfectant should achieve at least 3 log reduction for vegetative microorganisms and at least 2 log reduction for spore forming microorganisms mention in USP general chapter <1072> " Disinfectant And Antiseptics".

18.0 ABBREVIATIONS:

Abbreviations	Full Form
%	Percentage
v/v	Volume/volume
min.	Minute
LAF	Laminar Air Flow
ATCC	American Type Culture Collection
NCIM	National Collection of Industrial Microorganism
°C	Degree Celcius
ml	Mili liter
µ	Micron

19.0 LIST OF ATTACHMENTS:

Attachment No.	Title/ Details
1.	Certificate of Cultures
2.	Media GPT Record
3.	Sterilization Record
4.	Training Record
5.	Culture Suspension

20.0 REFERENCES:

Sr. No.	Title/ Details
1.	USP General Chapter <1072> "DISINFECTANTS AND ANTISEPTICS".
2.	SOP "SOP FOR GROWTH PROMOTION TEST OF CULTURE MEDIA" no. IIRT/SOP-MBD-004
3.	SOP "SOP FOR OPERATION AND CLEANING OF VERTICAL AUTOCLAVE" no. IIRT/SOP-MBD-016