PROTOCOL

FOR

EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Protocol No.	
Supersedes No.	Nil
Effective Date	

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

1.0 PROTOCOL APPROVAL:

This is a protocol to demonstrate the Efficacy Evaluation of Disinfectants and Sanitizing agents used at …….. the protocol has been prepared, reviewed and approved for execution by personnel from the following departments:

Microbiologist;

PREPARED BY	SIGNATURE	DATE

Department Head:

REVIEWED BY	SIGNATURE	DATE

Head Regulatory Affairs:

REVIEWED BY	SIGNATURE	DATE

Head Quality Assurance:

APPROVED BY	SIGNATURE	DATE

Head Production:

APPROVED BY	SIGNATURE	DATE

Quality Head:

APPROVED BY	SIGNATURE	DATE

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

2.0 PURPOSE:

This protocol is designed to establish the scientific evidence and demonstrate the efficacy of disinfectants and sanitizing agents against various microorganisms by "Use Dilution or Concentration Method" and "Surface Challenge Test." to establish the scientific evidence and demonstrate the contact time of the disinfectant and sanitizing agents for its application. To establish the scientific evidence and demonstrate the validity of the storage period for in use disinfectants and sanitizing agents.

3.0. SCOPE:

This protocol is applicable for the Efficacy Evaluation of Disinfectants and Sanitizing agents, Neutralization study of Disinfectants, Contact Time Establishment and its Hold Time Study for Expiry Date when used at as per supplier recommended dilutions for the routine sanitization and disinfection applications to control the microorganism at …….

4.0 RESPONSIBILITY:

4.1 **Microbiology Executive/Designee-** Preparation of validation protocol, Execution of the validation studies and Completion of the validation report.

4.2 **Head QC/Designee** – Responsible for review of the protocol and its summary report for execution of experimental validation study and arranging resources for the validation program and review of validation results and summary report.

4.3 **Head Production/Designee** – Responsible for review of the protocol and its summary report for any technical aspects on the evaluation study.

4.4 **Head QA/Designee** – Responsible for the final approval of the protocol and summary report, after completion of qualification summary report shall be Checked, Reviewed and Approved.

5.0 REFERENCES:

5.1 USP Chapter <1072> Disinfectant and Antiseptics.

6.0 METHODOLOGY:

6.1 Pre-Requisites

6.1.1 Media Required:

- Soyabean Casein Digest Agar.
- Sabouraud Dextrose Agar.
- Tryptone Soya Agar with Lecithin and Tween-80 or Dey/Engley (D/E) agar.
- 0.1% Peptone Water.

- Dey/Engley (D/E) Broth.
- 0.9% Saline

6.1.2 Accessories Required:

- Sterilized Membrane 0.45µm
- Filter Assembly
- Filter Cups
- Tubings
- Micropipette
- Microtips
- Forceps
- Petriplates
- Surface Test Coupons

6.2 Challenge Microorganisms:

6.2.1 Selection of ATCC and Environmental isolates is done to cover the entire microorganism based on the gram character and cell morphology.

6.2.2 For the studies on disinfectants used for spraying and surface disinfection isolates selected must involve spore bearing prokaryotic and eukaryotic microorganisms. The typical challenge microorganisms that can be employed are listed in Table-01.

Table-01

S.No.	Name of the challenge Microorganism (Standard Test Organism)	Disinfectant/Sanitizing Agent Used	Cell Morphology
1.	*Escherichia coli* (ATCC 8739)	Bactericide	Vegetative Bacteria and Gram –ve small bacilli
2.	*Staphylococcus aureus* (ATCC 6538)	Bactericide	Vegetative Bacteria and Gram +ve cocci in clusters.
3.	*Pseudomonas aeruginosa* (ATCC 9027)	Bactericide	Vegetative Bacteria and Gram –ve bacilli
4.	*Bacillus subtilis* (ATCC 6633)	Sporicide	Spore forming bacteria and Gram +ve bacilli.

S.No.	Name of the challenge Microorganism (Standard Test Organism)	Disinfectant/Sanitizing Agent Used	Cell Morphology
5.	*Candida albicans* (ATCC 10231)	Fungicide	Yeast, prokaryotic budding cells.
6.	*Aspergillus brasiliensis* (ATCC 16404)	Fungicide/Sporicide	Mold, Spore forming mycelium.
7.	Environment Isolates	Bactericide	Gram +ve cocci

6.2.3 However, all the environmental isolates, which were isolated from an environmental monitoring program shall be challenged to the efficacy evaluation of disinfectants/sanitizing agents to confirm their susceptibility, other wise most frequently isolated microorganisms is also acceptable.

6.3 Classification of Disinfectant and Sanitizing Agents:

6.3.1 Chemically Disinfectants are classified by their chemical type. These include Aldehydes, Alcohols, Halogens, Peroxides, Quaternary Compound (QAC) and Phenolic Compounds (see Table-02).

Table-02

Chemically Entity	Classification	Examples
Aldehydes	Sporicidal agents	2% Glutaraldehyde
Alcohols	General purpose disinfectant (Bactericide), antiseptic, antiviral agent	70% Isopropyl Alcohol (IPA)
Chlorine and Sodium hypochlorite	Sporicidal agent	0.5% Sodium hypochlorite.
Phenolics	General purpose disinfectant	500 µg per g chlorocresol, 500 µg per g chloroxylenol.
Ozone	Sporicidal agent	8% gas by weight.
Hydrogen peroxide	Vapour phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g H2O2 vapour, 10%-25% solution, 3% solution.
Substituted diguanides	Antiseptic agent	0.5% Chlorhexidine gluconate

Chemically Entity	Classification	Examples
Peracetic acid	Liquid sterilant, vapour phase sterilant	0.2% Peracetic acid, 1 µg per g Peracetic acid
Ethylene oxide	Vapour phase sterilant	600 µg per g Ethylene oxide.
Quaternary ammonium compounds	General purpose disinfectant (bactericide), antiseptic	200 µg per g Benzylkonium chloride.
B-Propiolactone	Sporicidal agent	100 µg per g B-Propiolactone

6.4 Selection Criteria of Disinfectants and Sanitizing Agents:

6.4.1 The Following points to be consider for selection of disinfectants and sanitizing agents.

- Number and types of microorganisms to be controlled.
- The spectrum of activity of commercially available disinfectants and sanitizing agents.
- The claims as a sterilant.
- The disinfectant or sanitizer supporters by the EPA registrations.
- The concentration, application method and contact time with the disinfectant or sanitizing agent.
- Nature of the surface material and its compatibility with the disinfectant or sanitizing agents.
- The amount of organic compounds on the surface that may inactivate a disinfectant or sanitizing agent.
- The possible need to maintain a residual bactericidal activity of the disinfectant on the surface.
- The corrosiveness of the disinfectant to equipment with repeated application.
- Safety consideration to the operators applying the disinfectant or sanitizing agents.

6.5 Neutralizing agents for Disinfectants and Sanitizing agents:

6.5.1 Neutralizers that inactivate the disinfectants shall be included either in the diluent or microbiological media used for microbial enumeration. Refer Table-03 for information on the disinfectant neutralization.

Table-03

Disinfectant	Neutralizing Agent
Alcohols,	Dilution or Polysorbate 80
Glutaraldehyde	Glycine and Sodium bisulfate
Sodium hypochlorite	Sodium thiosulphate
Chlorhexidine	Polysorbate 80 and Lecithin
Mercuric chlorides and other mercurials	Thioglycolic acid
Quartnery ammonium compounds	Polysorbate 80 and Lecithin
Phenolic compounds	Dilution or Polysorbate 80 and Lecithin
A universal neutralizing broth which contain a range of neutralizing agents can also be used for example Dey/Engley (D/E) broth which contains 0.5% Polysorbate 80, 0.7% Lecithin, 0.1% Sodium thioglycolate, 0.6% Sodium thiosulphate, 0.25% Sodium bisulfate, 0.5% tryptone, 0.25% yeast extract and 1.0% dextrose.	

7.0 Validation Procedure:

7.1 This validation methodology demonstrated for the following parameters:

- Preparation of challenge inoculum culture.
- Neutralization study of disinfectants and sanitizing agents.
- In vitro "Use dilution" test and contact time Establishment (screening disinfectants and sanitizing agents for their efficacy at various concentrations and contact times against a wide range of standard test organisms and environmental isolates).
- In vitro "Surface Challenge Test" on test coupons.

7.2 Preparation of challenge inoculum:

7.2.1 Prepare SCDA and SDA slants as per the current version of SOP "Preparation of Microbial Culture Suspension".

7.2.2 Incubation conditions for the slants is as follows:

- For Bacillus species incubate at 30 to 35 ºC for NLT 2 Days.
- For Vegetative growth of bacteria incubates at 30 to 35 ºC for NMT 3 Days.
- For Yeast incubate at 20 to 25 ºC for NLT 5 Days.
- For Molds incubate at 20 to 25 ºC for NLT 5 Days.

7.2.3 Confirm the purity check of the spore formation by spore staining method for all spore forming bacteria as per current version of SOP "Management of microbial cultures.

7.2.4 After completion of incubation period wash the slants with 0.9% saline to get uniform inoculum suspension. This shall be used to prepare the 10^6 cfu/ml and to obtain the NMT 100 cfu by serial dilution techniques.

7.2.5 Check the suspension for the cell population by culture suspension preparation method as per current version of SOP "Preparation of Microbial Culture Suspension".

7.2.6 Record the observations and results in the Annexure-1

7.3 Neutralization study of Disinfectants and Sanitizing Agents:

7.3.1 This test method is used to establish and demonstrate the elimination or minimizing the chemical effect of the disinfectant on the microbial population when performing the recovery test.

7.3.2 Sample Preparation:

7.3.2.1 Prepare Culture suspension as per the current version of SOP "Preparation of Microbial Culture Suspension" to determine NMT 100 cfu concentration of the following microorganisms selected:

- *Escherichia coli* (ATCC8739)
- *Staphylococcus aureus* (ATCC6538)
- *Candida albicans* (ATCC10231)
- *Aspergillus brasiliensis* (ATCC16404)
- *Pseudomonas aeruginosa* (ATCC 9027)
- *Bacillus subtilis* (ATCC 6633)
- Environment Isolates

7.3.2.2 Prepare "Use Dilution" in a separate tube of each disinfectant and sanitizing agent used for the study.

7.3.2.2 Prepare and sterilize the required quantity of neutralizing media in separate container, dispense 50 ml of the media into 100 ml test tubes for each challenge microorganisms.

7.4 List of Disinfectant and Sanitizing Agents.

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S.No.	Name of Disinfectant	Use Concentration	Category
1.	Bacillocid	2.0 %	Bactericidal, Sporicidal
2.	Taski Combaton DS	1.0 %	Bactericidal, Sporicidal
3.	Virosil	20 %	Sporicidal
4.	Sterillium	Undiluted	Bactericidal, Sporicidal
5.	Isopropyl alcohol	70 %	Bactericidal, Sporicidal
6.	Divosan (Clearklens Activ)	1 %	Sporicidal
7.	Virex II 256	0.5%	Bactericidal, Fungicidal, Virocidal
8.	Oxivir 516	1%	Bactericidal, Fungicidal, Virocidal

7.5 Use Dilution Test and its contact time Establishment:

7.5.1 This method involves the screening disinfectants for their efficacy at various concentrations and contact times against a wide range of standard microorganisms and environmental isolates.

7.5.2 Sample Preparation:

7.5.2.1 Dilute the sanitizers with sterile purified water at ambient temperature for the stock according to the recommendation of the manufacturer. This is termed as "Use Dilution"

7.5.2.2 Prepare the "Use Dilution" of each disinfectant.

7.5.2.3 Dispense 20ml of the dilution into four sterile test tubes and label them as A, B, C and D. A for 0 Min., B for 5 min., C for 10 min. and D for 15 min.

Note: Label on Sterillium or IPA 70% (Hand sanitizer) study tubes as A for 10 Seconds, B for 20 Seconds, C for 30 Seconds and D for 60 Seconds and note down the observations in Annexure-5 and calculate the log reduction.

7.5.2.4 Add 0.1 ml of culture containing 10^8 cfu/ml (for Bacteria & Yeast) or 10^5 cfu/ml (for Mold) of specified microorganism into tubes to get the concentration of $1x10^8$ cfu/ml (for Bacteria & Yeast) and $1x10^5$ cfu/ml (for Mold) sample A, B, C and D.

7.5.3 Recovery Study from Disinfectant Control Test:

7.5.3.1 Within 1 minute (0 minute) contact time immediately transfer the sample from A (20ml) into the 50 ml of diluents containing neutralizer.

7.5.3.2 Filter the solution through 0.45µm sterile membrane and give three rinses of 100 ml each with 0.1% peptone or suitable diluents.

7.5.3.3 Place a membrane aseptically on pre-incubated agar plates (TSA with Lecithin & Polysorbate 80) or plates containing neutralizer (DE agar) according to the nature of the disinfectants.

7.5.3.4 Repeat the above exercise to establish the contact time for 5 minutes, 10 minutes and 15 minutes for tubes B, C & D respectively for each sanitizing agent used.

7.5.4 Recovery Study from Positive Control Test:

7.5.4.1 Add 0.1 ml of the challenge microorganism containing 10^8 cfu/ml (for Bacteria & Yeast) or 10^5 cfu/ml (for Mold) to 20 ml sterile normal saline, further transfer to 50 ml neutralizing media, filter the solution through 0.45 µm sterile membrane and place the membrane on the pre-incubated TSA or DE agar plate aseptically.

7.5.4.2 Repeat the procedure for all specified microorganisms.

7.5.4.3 Incubation Conditions: Bacteria: 30 to 35 ºC for 48 to 72 hrs.

Yeast & Mold: 20 to 25 ºC for 5days.

7.5.4.4 Interpretation of Results: After the incubation period, count the number of colonies on the membrane from all plates and note down the observation in Annexure-2 and calculate the log reduction.

7.5.5 Negative Control:

7.5.5.1 Filter 20 ml sterile normal saline in 50 ml neutralizing media, filter the solution through 0.45 µm sterile membrane filter and place the membrane on the pre-incubated TSA or DE agar plate aseptically..

7.5.5.2 Incubate the plates at 20-25 ºC for 72 hrs. Followed by 30-35 ºC for 48 hrs.

7.5.6 Log Reduction Calculation:

(**X**-Y)

Where, X = Log of population taken for test.

Y = Log of observed count after contact time.

7.6. Description:

7.6.1. This method involves using standard test microorganisms and microorganisms that are typical environmental isolates, applying disinfectants to the selected surface at the “Use Dilution” concentration with a specified contact time, and determined the log reduction of the challenge microorganisms. This is considered necessary because critical process steps like disinfection of aseptic processing area, as required by GMP regulation, needed to be validated, based on surface application.

7.6.2 Selection Criteria of Surface:

7.6.2.1 All the surface base on the disinfection application criteria.

7.6.2.2 Because a wide range of different material of construction are used in the clean rooms and other controlled areas each material need to be evaluated separately to validate the efficacy of the given disinfectant and sanitizing agents. Also contains the common materials used in the clean room construction as per Table-04 given below

Table-04

Material	Application
Stainless Steel	Work Surface, Filling Equipment and Tank
Glass	View Panels
Epoxy	Floor
Kota Stone	Floor
Clestra Panels	Wall Panels

7.6.3 Sample Preparation:

7.6.3.1 Surface coupons like S.S., Glass & Kota Stone shall be wrap by aluminum foil and sterilized in steam sterilizer and Epoxy shall be surface sanitized with 20% Virosil.

7.6.3.2 After the sterilization carry the S.S., Glass and other surface coupons into the LAF area and unwrap coupons carefully before analysis.

7.6.4 Recovery from Test Surface:

7.6.4.1 Select Three areas of 2 inch x 2 inch square on one coupon surface. 1st area shall be used for test sample surface recovery, 2nd shall be used for positive test surface recovery and 3rd shall be used as a negative control.

7.6.4.2 Add 0.1ml of the cell suspension containing approximately 10^8 cfu/ml (for Bacteria & Yeast) or 10^5 cfu/ml (for Mold) of any one selected microorganism on the template surface area (1st and 2nd) and spread equally with an L-spreader.

7.6.4.3 Hold the coupon in vertical position and apply selected disinfectant by fine spray on the spike surface area of the template surface.

7.6.4.4 Take precaution not to over spill the applied disinfectant to other marked surface.

7.6.4.5 Allow the disinfectant as per established contact time on the template surface and recover by challenge inoculums by swab method on the three surfaces with individual swab sticks.

7.6.4.6 Rotate and spread the swab throughout the selected surface (1st area of the template surface) in zigzag fashion.

7.6.4.7 Place the swab immediately in to a tube containing 10 ml Neutralizing broth.

7.6.4.8 Repeat the procedure for all specified microorganisms and with each selected disinfectant on each mentioned surface coupons respectively (0 Minute, 5 Minutes, 10 Minutes & 15 Minutes).

Note: In case of Hand sanitizer (Sterillium or IPA 70%) the study will be performed on 10, 20, 30 & 60 Seconds and note down the observations in Annexure-6 and calculate the log reduction.

7.6.5 Recovery from Control Surface:

7.6.5.1 For control surface specimen spike the 0.1 ml of the cell suspension containing approximately 10^8 cfu/ml of $1x10^8$ concentration culture spread equally for the 2 x 2 inch area.

7.6.5.2 Take the swab from 2nd area of the template surface similar to the specimen; place the swab into the neutralizing media culture.

7.6.6 Recovery from control surface:

7.6.6.1 3rd part of the selected coupon surface area of 2 x 2 inch area which is not spike with microorganism and disinfectant treated as test negative control.

7.6.7 Swab Test Procedure:

7.6.7.1 Vortex the tube containing swab for about 30 second and proceed by filtration method.

7.6.7.2 Arrange filter assembly, attach the vacuum pump and filter the Neutralizing broth tube through 0.45µm x 47mm membrane and aseptically transfer the membrane on pre-incubated TSA or DE agar plate for bacterial cultures and yeast and mold culture.

7.6.8 Incubation Conditions:

7.6.8.1 Bacteria: 30 to 35 ºC for 48 to 72 hrs.

Yeast & Mold: 20 to 25 ºC for 5days.

7.6.9 Interpretation of Results:

7.6.9.1 After the incubation period count the number of colonies on the membrane from all plates and note down the observations in Annexure-4 and calculate the log reduction.

7.7 Hold Time Establishment Study:

7.7.1 To determine the validity of the disinfectant for the certain period of storage time in use container for the regular application shall be demonstrated for its effectiveness when compared to initial day of preparation.

7.7.2 Following parameters are established to demonstrate the effectiveness.

- Efficacy study after 2nd day.
- Bio burden level of the disinfectant.

7.7.3 Sample Preparation:

7.7.3.1 Dilute all sanitizing agents which were used in the contact time establishment study with sterile purified water at ambient temperature from the stock according to the recommendations of the manufacturer. This is termed as “Use Dilution”

7.7.3.2 Use the same dilution which was established in the "Use Dilution Test" (In contact time establishment study).

7.7.3.3 Prepare the "Use Dilution" of all the disinfectants and sanitizing agents used of 20 ml quantity.

7.7.3.4 Prepare the culture suspension to have $1x10^8$ (for Bacteria & Yeast) or $1x10^5$ cfu/ml (for Mold) populations. Refer preparation of challenged inoculum procedure of this protocol.

7.7.3.5 Prepare 20ml of neutralizing media solution in the test tubes for all the challenged microorganisms.

7.7.4 Recovery from Test Sample by Use Dilution Method:

7.7.4.1 Add 0.1ml culture suspension of challenged microorganisms in each 20 ml disinfectant.

7.7.4.2 Hold the diluted disinfectant for 2 days in the aseptic area or in the controlled area.

7.7.4.3 Arrange the filter assembly in the LAF, connect to the vacuum pump and filter the content of each selected dilution through separate 0.45 µm x 47 mm membrane filter.

7.7.4.4 Aseptically transfer the membrane filter on pre-incubated media plates of sterile TSA for bacterial cultures and yeast & mold cultures.

7.7.4.5 Recovery study shall be performed at 2^{nd} day hold time.

7.7.5 Recovery from Positive Control Sample:

7.7.5.1 Add 0.1ml of challenged microorganisms in each 20 ml neutralizing media solution.

7.7.5.2 Arrange the filter assembly on the LAF, connect to the vacuum pump and filter the content of each prepared above solution through 0.45 µm x 47 mm membrane filter.

7.7.5.3 Aseptically transfer the membrane filter on pre-incubated media plates of sterile TSA for bacterial culture and yeast & mold cultures.

7.7.5.4 Positive control recovery is performed along with the test control.

7.7.6 Negative Control:

7.7.6.1 Only neutralizing media shall be used for negative control test. Filter the whole 20 ml content of the neutralizing media through 0.45 µm membrane.

7.7.6.2 Aseptically transfer the membrane on the pre-incubated TSA plate and incubate.

7.7.7 Incubation Condition:

- Bacteria- 30-35 °C for 48 to 72 hrs.
- Yeast & Mold - 20-25 °C for 48 to 5 days.
- Negative Control- 20-25 °C for 72 hrs. Followed by 30-35 °C for 48 to 72 hrs.

7.7.8 Interpretation of Results:

7.7.1 After the incubation period count the number of colonies on the membrane from all plates and note down the observation in Annexure– 4 and 5.

7.8 Bio burden Test:

7.8.1. Sample Preparation:

7.8.1.1. Take aseptically 1 ml of manufacturer recommendation concentration of the disinfectant and transfer in sterile petri plate in duplicate for Total bacterial count and Total fungal count.

7.8.1.2. Pour approximately 15-20 ml of sterile DE agar in each plate and mix properly by rotating the plate clockwise and anti-clockwise direction and allow them for solidification.

7.8.2. Negative Control:

7.8.2.1. Only neutralizing media shall be used for negative control.

7.8.3. Incubation Condition:

- Bacteria- 30-35 °C for 48 to 72 hrs.
- Yeast & Mold - 20-25 °C for 48 to 5 days.
- Negative Control- 20-25 °C for 72 hrs. Followed by 30-35 °C for 48 to 72 hrs.

7.9 Acceptance Criteria:

7.9.1 For contact time establishment there should be minimum 5 log reduction for vegetative bacteria/ yeast and 3 log reduction for bacteria spore/fungi (mold) with a control disinfectant and sanitizing agents.

7.9.2 For surface coupons studies there should be minimum 3 log reduction for vegetative bacteria/ yeast and 2 log reduction for bacteria spore/fungi (mold) with a control disinfectant application.

7.9.3 The 2 day hold time study disinfectant in use studies for established contact time should show 5 log reduction for vegetative bacteria/ yeast and 3 log reduction for bacterial spore/fungi (mold) with a control disinfectant and sanitizing agents stored.

7.9.4 There should not be any microbial growth in store disinfectant to establish with selected hold time period.

8.0 SUMMARY OF DEVIATIONS:

8.1 Any deviation(s) from the protocol while performing the methodology shall be investigated and documented in the report.

9.0 ABBREVIATIONS:

9.1 QC – Quality Control

9.2 QA- Quality Assurance

9.3 SCDA- Soyabean Casein Digest Agar

9.4 SDA- Sabouraud Dextrose Agar

9.5 TSA- Tryptic Soya Agar

9.6 ATCC- American Type Culture Collection

9.7 CFU- Colony Forming Units

9.8 NMT- Not More Than

9.9 NLT- Not Less Than

9.10 LAF- Laminar Air Flow

10.0 DOCUMENTATION AND ARCHIVAL:

10.1 **Report:** At the end of the study a report shall be prepared.

10.2 **Archival:** The original and executed document shall be hand it over to QA for archival.

11.0 ANNEXURES:

11.1 **Annexure-1:** Inoculum Preparation Record.

11.2 **Annexure-2:** Growth Observation Record for Direct Contact Method.

11.3 **Annexure-3:** Surface Challenge Test.

11.4 **Annexure-4:** Hold Time Establishment by Use Dilution Method.

11.5 **Annexure-5:** Growth observation Record of Hand Sanitizer for Direct Contact Method

11.6 **Annexure-6:** Growth observation Record of Hand Sanitizer for Surface Challenge Test

11.7 **Annexure-7:** Bio burden Test Report of Disinfectant and Sanitizing Agents.

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ANNEXURE- 1
INOCULUM PREPARATION RECORD

Name Organism		ATCC No.	
Date of Preparation		Date of Reporting	
Media Used		Media Lot. No.	
Incubation Temperature		Incubator ID	
From		To	
Reference Protocol No.:			

Dilution	Volume Tested	Count /Plate			Final Inoculum Population	Observed By (Sign / Date)
		Plate 1	Plate 2	Average		

Remarks:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)

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ANNEXURE- 2

GROWTH OBSERVATION RECORD FOR DIRECT CONTACT METHOD

Protocol No.:……

Name of Disinfectant		**Disinfectant Batch No.**	
Concentration		**Date of Analysis**	
Name of Media		**Media Lot. No.**	
Neutralizing Diluents Used		**Lot No. of Diluents**	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		**Incubator ID**	
Incubation Temperature	22.5±2.5 ºC	**Incubation Temperature**	32.5±2.5 ºC
From		**From**	
To		**To**	

Name of Organism	**Initial Population CFU/ml**	**Volume Tested**	**Population taken for Test (X)**	**Observed count after contact time(Y)**				**Log reduction Observed (X-Y)**				**Observed By (Sign /Date)**
				0 Min.	**5 Min.**	**10 Min.**	**15 Min.**	**0 Min.**	**5 Min.**	**10 Min.**	**15 Min.**	
Bacillus subtilis												
Escherichia coli												
Staphylococcus aureus												
Candida albicans												
Aspergillus brasiliensis												
Pseudomonas aeruginosa												
Environmental Isolate-1												
Environmental Isolate-2												
Environmental Isolate-3												

Remarks:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)

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ANNEXURE III
SURFACE CHALLENGE TEST

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: 20-25ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Bacillus subtilis	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra panels												

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Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Escherichia coli	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Staphylococcus aureus	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: 20-25ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Pseudomonas aeruginosa	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Candida albicans	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Aspergillus brasiliensis	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Environmental Isolate-1	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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MICROBIOLOGY DEPARTMENT

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: 20-25ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Environmental Isolate-2	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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MICROBIOLOGY DEPARTMENT

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign/Date)
					0 Min.	5 Min.	10 Min.	15 Min.	0 Min.	5 Min.	10 Min.	15 Min.	
Environmental Isolate-3	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Remarks:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

ANNEXURE- 4

HOLD TIME ESTABLISHMENT BY USE DILUTION METHOD

Protocol No.: ……

Name of Disinfectant		Disinfectant Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Initial Population CFU/ml	Volume Tested	Population taken for Test (X)	Observed count After 2 Days(Y)	Log reduction observed after 2 days (X-Y)	Observed by (Sign /Date)
Bacillus subtilis						
Escherichia coli						
Staphylococcus aureus						
Pseudomonas aeruginosa						
Candida albicans						
Aspergillus brasiliensis						

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Organism	Initial Population CFU/ml	Volume Tested	Population taken for Test (X)	Observed count After 2 Days(Y)	Log reduction observed after 2 days (X-Y)	Observed by (Sign /Date)
Environmental Isolate -1						
Environmental Isolate -2						
Environmental Isolate-3						

Remarks:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

ANNEXURE- 5

GROWTH OBSERVATION RECORD OF HAND SANITIZER FOR DIRECT CONTACT METHOD

Protocol No.:

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	**Initial Population CFU/ml**	**Volume Tested**	**Population taken for Test (X)**	**Observed count after contact time(Y)**				**Log reduction observed (X-Y)**				**Observed by (Sign /Date)**
				10 Sec.	**20 Sec.**	**30 Sec.**	**60 Sec.**	**10 Sec.**	**20 Sec.**	**30 Sec.**	**60 Sec.**	
Bacillus subtilis												
Escherichia coli												
Staphylococcus aureus												
Candida albicans												
Aspergillus brasiliensis												
Pseudomonas aeruginosa												
Environmental Isolate-1												
Environmental Isolate-2												
Environmental Isolate-3												

Remarks:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

ANNEXURE- 6
GROWTH OBSERVATION RECORD OF HAND SANITIZER FOR SURFACE CHALLENGE TEST

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Bacillus subtilis	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Escherichia coli	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Staphylococcus aureus	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Pseudomonas aeruginosa	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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MICROBIOLOGY DEPARTMENT

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Candida albicans	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Aspergillus brasiliensis	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	**Surface**	**Initial Population cfu/ml**	**Volume Tested**	**Population Taken for Test cfu/ml (X)**	**Observed count after contact time (Y)**				**Log reduction Observed (X-Y)**				**Observed by (Sign./Date)**
					10 Sec.	**20 Sec.**	**30 Sec.**	**60 Sec.**	**10 Sec.**	**20 Sec.**	**30 Sec.**	**60 Sec.**	
Environmental Isolate-1	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Environmental Isolate-2	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

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PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

Name of Hand Sanitizer		Hand Sanitizer Batch No.	
Concentration		Date of Analysis	
Name of Media		Media Lot. No.	
Neutralizing Diluents Used		Lot No. of Diluents	

Incubation Details: - 20-25 ºC for 5 Days and 30-35ºC for 3 Days

Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC
From		From	
To		To	

Name of Organism	Surface	Initial Population cfu/ml	Volume Tested	Population Taken for Test cfu/ml (X)	Observed count after contact time (Y)				Log reduction Observed (X-Y)				Observed by (Sign./Date)
					10 Sec.	20 Sec.	30 Sec.	60 Sec.	10 Sec.	20 Sec.	30 Sec.	60 Sec.	
Environmental Isolate-3	SS												
	Epoxy												
	Glass												
	Kota Stone												
	Clestra Panels												

Remarks:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)

PROTOCOL FOR EFFICACY EVALUATION OF DISINFECTANTS & SANITIZING AGENTS

ANNEXURE -7

BIOBURDEN TEST REPORT OF DISINFECTANT AND SANITIZING AGENTS

Protocol No.:

Name of Disinfectant		Lot No./Batch No.	
Analysis Start on		Analysis Completion On	
Name of Media		Media Lot. No.	
Incubator ID		Incubator ID	
Incubation Temperature	22.5±2.5 ºC	Incubation Temperature	32.5±2.5 ºC

Observations

0 Day						7 Days						15 Days						30 Days					
TBC			TFC			TBC			TFC			TBC			TFC			TBC			TFC		
Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.	Plate 1	Plate 2	Avg.

Negative Control:
Remarks:

Positive Control:

Done by:
(Sign/Date)

Checked by:
(Sign/Date)