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A RESEARCH: EXAMINATION, VALIDATION AND EFFICACY TESTING OF THE DISINFECTANT USED IN STERILE MANUFACTURING UNIT

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ABSTRACT

Objective: Cleaning as well as disinfection of surfaces are important steps for maintaining and controlling the sterility of pharmaceutical manufacturing operations. The disinfectants and antiseptics are used to control the growth of micro-organisms thereby reducing the contamination in the final product. One of the more tedious tasks for pharmaceutical organizations is to deal with the selection of disinfectants and ensuring that the disinfectants choice are of desired characteristics and that the effectiveness of the disinfectants are periodically assessed. In this study the disinfectant (TOTASEP) was examined and validated for its use in sterile manufacturing facility. The validation was based upon the regulation guidelines provided by W.H.O and USP. **Methods:** There were three methods used to investigate the efficacy of the disinfectants, which were membrane filtration and direct inoculation method, swab analysis method and agar diffusion also known as ditch plate method. **Results:** These methods demonstrated that the disinfectant was active against the standard bacteria and fungi. TOTASEP 1%v/v solution showed its action within 10 minutes in both membrane filtration as well as swab analysis. Agar diffusion method resulted in a perfect zone of inhibition for the respective disinfectant. **Conclusion:** The validation of disinfectant was achieved according to the procedures provided in IP and W.H.O guidelines. The results from different

methods were found to be promising and the process was validated and the disinfectant was found to be efficient for the desired purpose.

KEYWORDS: Validation, Disinfectant, Antimicrobial agent, Efficacy, Microbial Contamination.

INTRODUCTION

Antiseptics as well as disinfectants are used widely in hospitals and also in other health care centers as well as in pharmaceutical industries to majorly control and prevent the growth of microbes on living tissues as well as on inanimate objects.[1,3,6] It is also used to prevent the contamination of the pharmaceutical preparations by various organisms with the aid of using it at various concentrations. These are important constituents of infection control methods which are used in the prevention of nosocomial infections.[1] But the general challenge is the choice of appropriate and desirable disinfectants as well as antiseptics because different pathogens gives variety of response to different antiseptics as well as disinfectants.[2,5] A variety of methods are accessible for studying the mechanisms of action of antiseptics as well as disinfectants on microorganisms, especially bacteria. The disinfectant acts by following mechanisms which causes death of microbes including, lysis followed by leakage of intracellular constituents, perturbation of cell homeostasis, modification of membrane characteristics, inhibition of enzymes, electron transport, oxidative phosphorylation, interaction with macromolecules, effects on macromolecular biosynthetic processes, and interaction with the genetic materials.[7,13] Many of these actions are informative for detecting as well as evaluating antiseptics or disinfectants used in combination. “Biocide” is a general term which portrays a chemical agent, normally broad spectrum that inactivates microorganisms.[8] Since biocides range in antimicrobial activity, other terms can be more specific, which includes “static,” which states the agents which inhibit growth (e.g. bacteriostatic, fungistatic and sporistatic) and “cidal” reflecting to agents which kill the target organism (e.g. sporicidal, virucidal, and bactericidal).[9]

Antiseptics are biocides or agents which destroy or inhibit the growth of microorganisms in or on living tissue (e.g. health care personnel hand washes and surgical scrubs) and disinfectants are nothing but similar instead are products or biocides that are used to sterilize inanimate objects or surfaces.[2,8] Disinfectants may be sporostatic but are not usually sporicidal. Sterilization refers to a physical or chemical process that completely destroys or removes all microbial life, involving spores too. While preservation is the prevention of

growth of microorganisms in formulated products, including pharmaceuticals and foods.[5,11] Variety of biocides are used for cleaning purposes, cleaning in these cases reflects to the physical removal of foreign material from a surface.

- **Characteristics of an Ideal Disinfectant[12]**

1) They should be microbicidal.
2) It must be easy to use.
3) It should comprise of detergent activity.
4) These should non – toxic and should require minimum safety controls.
5) These should be non-irritating.
6) Disinfectants used should be harmless to surfaces. Which means they should be corrosion free.
7) Should possess rapid action property.
8) Should comprise activity in presence of organic matter.
9) Should have activity in presence of hard water.
10) Should be Stable at varying temperature and conditions.
11) Residual activity.
12) Should be inexpensive.

Factors Affecting the Disinfectant Efficacy.[1,8]

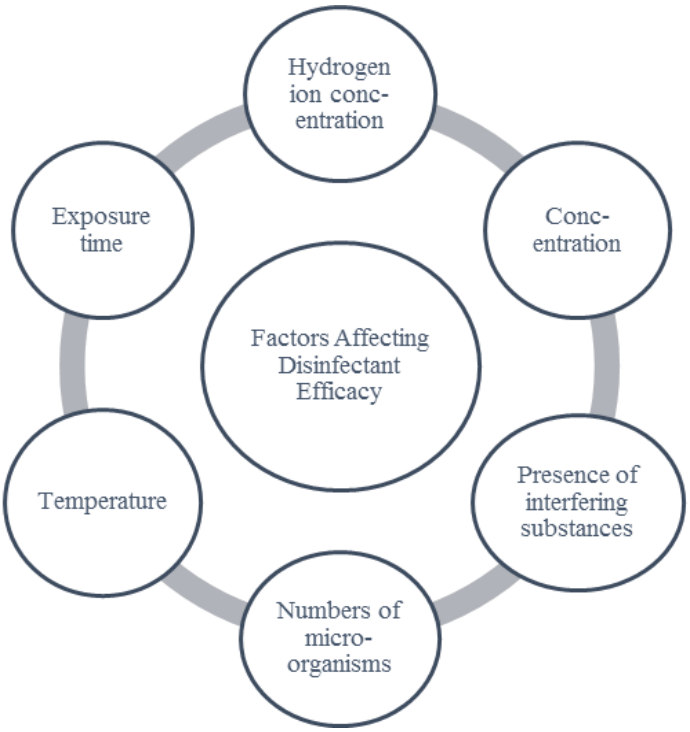

Fig No 1: Factors Affecting Disinfectant Efficacy.

MATERIALS AND METHODS

Bacterial Strains

S. aureus (6538P), E. coli (4157), P. aeruginosa (15442), B. subtilis (6633), S.abony (6017), A. brasiliensis(16404), C. albicans(10231).

Bacterial Growth Media

Soyabean Casein Digest Agar (SCDA), Soyabean Casein Digest Medium, Sabouraud Dextrose Agar with Chloramphenicol (SDA), Cetrimide Agar, Pseudomonas Agar Medium for Detection of Pyocyanin (PPA), Pseudomonas Agar Medium for Detection of fluorescein (PPF), Mannitol Salt Agar (MSA), MacConkeys Agar (MA), Fluid Thioglycolate Medium (FTM), Xylose-Lysine Deoxycholate Agar (XLDA), Wilson and Blair's BBS Agar (WBBS), Sterile Cotton Swabs in Screw capped.

Instruments used

Hot air oven (Modern Industrial Co-operation), Autoclave (Modi Enterprises Corporation), Laminar air flow (Kleanzone Systems India Private Limited), weighing balance(Mettler Toledo), heating mantle (Bio Technics India).

Fig No 2: Image of the disinfectant used.

- **Procedure for Serial Dilution of Bacterial and Fungal cultures[9]**

1. Aseptically transfer 1 ml of above prepared bacterial culture suspension of *E. coil (ATCC 4157)* to the vial containing 9ml of sterile saline. This corresponds to 10^{-1} dilution.

2. Aseptically mix the content well. Transfer 1ml of the dilution 10^{-1} to the vial containing 9ml of sterile saline. This corresponds to 10^{-1} dilution.
3. Continue the procedure to make further dilution viz. 10^{-3}, 10^{-4} up to 10^{-8}.
4. Repeat steps from 1 to 3 for the serial dilutions remaining bacterial cultures viz. *S. aureus (ATCC 6538 P), P. aeruginosa (ATCC 15442), B. Subtilis (ATCC 6633) and fungal cultures viz. C. albicans (ATCC 10231)* and *A. Niger (ATCC 16404).*

- **Test Procedures (Bactericidal and Fungicidal Activity)[6,8,15]**

1. Place 2.5 ml of undiluted disinfectant into sterile test tubes separately for each of the dilution of *E. coil (ATCC 4157)* viz. 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}cultures.
2. Suspended 0.25ml of the suspension of each dilution viz. 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} of *E. coil (ATCC 4157)* individually in each tube containing undiluted disinfectant (TOTASEP).
3. After specified time intervals viz. 10 mins, 20 mins, 30 mins, 40 mins and 50 mins inoculate 0.25 ml of the test suspension (micro-organism + disinfectant) from each vial of step 2 to individual vial containing 20 ml of molten Sterile Plate Count Agar (PCA) media.
4. Mix the media and culture properly, aseptically pour into individual sterile petri plates and allow it to solidify completely.
5. Repeat step 1-4 for remaining bacterial cultures viz. *S. aureus (6538P), E. coli (4157), P. aeruginosa (15442), B. subtilis (6633), S.abony (6017).*
6. Repeat steps 1 to 2 for fungal cultures viz. *C. albicans (ATCC 10231*) and *A. Niger (ATCC 16404).*
7. After specified time intervals viz. 10 mins, 20 mins, 30 mins, 40 mins and 50 mins inoculate 0.25 ml of the test suspension (micro-organism + disinfectant) from each vial of step 6 to individual vial containing 20 ml of molten Sterile Potato Dextrose agar (PDA) media.
8. Mix the media and culture properly, aseptically pour into individual sterile petri plates and allow it to solidify completely.
9. Incubate the plates viz. 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, $10^{-7}$of *S. aureus (6538P), E. coli (4157), P. aeruginosa (15442), B. subtilis (6633), S.abony (6017)* at 37°C for 72 hours.

10. Incubate the plate viz. 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} of C. albicans (ATCC 10231) at (25-30) ° C for 120 hours and the plates of A. brasiliensis (ATCC 16404) at (25-30) ° C for 168 hours.
11. Repeat step 1 to 10 using 0.5% v/v, 1% v/v and 1.5% v/v dilutions of TOTASEP.
12. Observe the plates to check the colony count.
13. For bacterial cultures run the positive control by individually inoculating 0.25ml of each dilution viz 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, $10^{-7}$of *E. coli* (ATCC 4157), *S .aureus* (ATCC 6538P), *P. aeruginosa* (ATCC 15442), *B. subtilis* (ATCC 6633), *S.abony(6017)* directly in 2.5ml of 0.9% sterile saline instead of the disinfectant and further inoculating 0.25ml of this in the vials containing 20ml of sterile Plate Count Agar (PCA) media. Mix the media and culture properly. Aseptically pour into individual sterile petri plates and allow it to solidify completely.
14. For fungal cultures run the Positive Control by individually inoculating 0.25ml of each dilutions viz. 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, of. *C. albicans (ATCC 10231),* and *A. brasiliensis (ATCC 16404)* directly in 2.5ml of 0.9% Sterile saline instead of the disinfectant and further inoculating 0.25ml of this in the vials containing 20ml of Sabouraud Dextrose Agar (SDA) media. Aseptically mix the media and culture properly and pour into individual sterile petri plates and allow it to solidify completely.
15. Incubate the plates of bacterial culture as mentioned in the step 9 and fungal cultures mentioned in step 10.
16. Observe the plates to check the colony count.
17. Run the Negative Control by inoculating 250ul of 0.9% Sterile Saline used for the test in 20ml of Sterile Plate Count Agar (PCA) media and 20ml of Sterile Sabouraud Dextrose Agar (SDA) media.
18. Mix the contents properly and aseptically pour into individual sterile petri plates and allow it to solidify completely.
19. Incubate the Plate Count Agar of Negative Control at 37^{0}C for 72 hours, the Potato Dextrose Agar plate of Negative Control at (25-30) ^{0}C for 168 hours.
20. Observe the Negative Controls for any Bacterial and Fungal Contamination.

$$\text{Viable Count} = \frac{\text{Average number of colonies X Dilution Factor}}{\text{Amount of Inoculum (0.25ml)}}$$

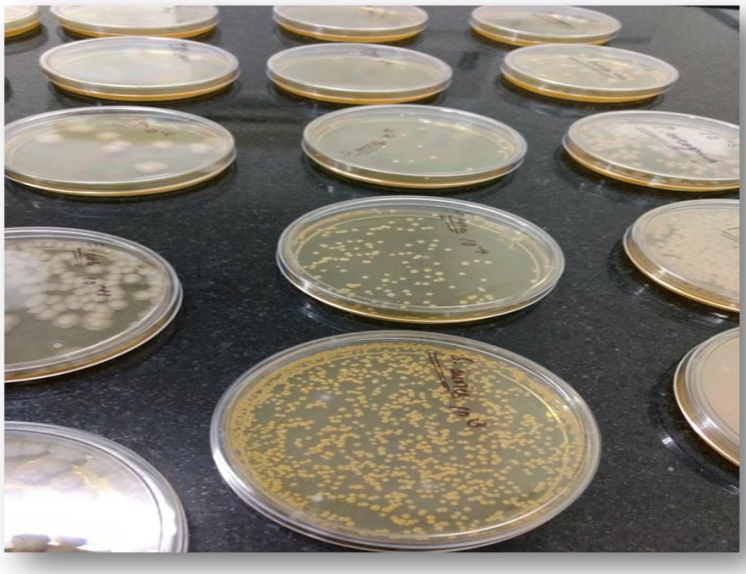

Fig No 3: viable count detection of micro-organisms.

RESULTS AND DISCUSSION

- **Positive control**

Table no 1: - Results without disinfectant after incubation at 37°C for 72 Hrs.

Micro-organisms	ATCC No.	Dilutions								Viable Count(CFU/ml)
		10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
E. coli	4157	M	M	UC	UC	UC	129	15	03	7.72×10^{-7}
S. aureus	6538P	M	M	UC	UC	UC	118	23	02	7.30×10^{-7}
P. aeruginosa	15442	M	M	UC	UC	UC	101	25	03	8.68×10^{-7}
B. subtilis	6633	M	M	UC	UC	UC	130	22	03	8.65×10^{-7}
S. abony	6017	M	M	UC	UC	UC	93	10	05	9.24×10^{-7}

Table no 2: Result after incubation at 25°c - 30°c for 120 Hrs.

Micro-organisms	ATCC No.	Dilutions								Viable count(CFU/ML)
		10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
C. albicans	10231	M	M	UC	UC	UC	105	09	04	7.93×10^{-7}

Table no 3: Result after incubation at 25°c - 30°c for 168 Hrs.

Micro-organisms	ATCC No.	Dilutions								Viable count(CFU/ml)
		10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
A. brasiliensis	16404	M	M	UC	UC	UC	102	22	04	9.62×10^{-7}

M=MATT GROWTH UC=UNCOUNTABLE CFU=COLONY FORMING UNITS

- **With disinfectant – TOTASEP (test) with 20 min. contact time.**

Table no 4: Result after incubation at 37°c for 72 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
E. coli	4157	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
S. aureus	6538P	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
P. aeruginosa	15442	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
B. subtilis	6633	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
S. abony	6017	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	

Table no 5: Result after incubation at 25°c - 30°c for 120 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
C. albicans	10231	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	

Table No.6: Result after incubation at 25°c - 30°c for 168 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
A.brasiliensis	16404	0.5	0	0	0	0	0	0	0	0	7 log reduction/30 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	

As the above results shows that the growth of micro-organism had been ceased by **TOTASEP** solution, now we need to check the lower limit of the time required by **TOTASEP** to show its bactericidal and fungicidal effect.

With disinfectant – TOTASEP (test) with 10 min. contact time

Table no 7: Result after incubation at 37°c for 72 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
E. coli	4157	0.5	UC	UC	121	54	19	7	1	0	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
S. aureus	6538P	0.5	UC	UC	119	45	21	9	3	0	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
P. aeruginosa	15442	0.5	UC	25	141	65	31	16	5	1	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
B. subtilis	6633	0.5	UC	UC	123	53	22	9	1	0	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	
S. abony	6017	0.5	UC	103	52	21	7	3	1	0	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	

Table no 8: Result after incubation at 25°c - 30°c for 120 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time
			10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
C. albicans	10231	0.5	UC	110	52	24	11	3	1	0	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	

Table no 9: Result after incubation at 25°c - 30°c for 168 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
A.brasiliensis	16404	0.5	UC	UC	54	19	6	2	1	0	7 log reduction/10 mins
		1	0	0	0	0	0	0	0	0	
		1.5	0	0	0	0	0	0	0	0	

As the above results shows that the growth of micro-organism had been ceased by 1% v/v Totasep solution, now we need to check the lower limit of the time required by Totasep at 1% v/v to show its bactericidal and fungicidal effect.

Here the concentration has been fixed at 1% v/v but the activity of Totasep (1% v/v) below 10 min. is needed to be checked to determine the end point.

With disinfectant – TOTASEP (test) with 5 min. contact time

Table no 10: Result after incubation at 37°c for 72 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
E. coli	4157	0.5	M	UC	143	108	51	20	8	3	-
		1	UC	UC	112	86	44	16	5	2	
		1.5	UC	UC	100	58	32	12	3	1	
S. aureus	6538P	0.5	M	UC	129	115	82	45	23	9	-
		1	UC	UC	112	93	75	33	18	2	
		1.5	UC	UC	92	76	51	27	11	5	
P. aeruginosa	15442	0.5	M	UC	136	111	87	45	13	4	-
		1	UC	UC	113	92	44	30	13	5	
		1.5	UC	UC	100	80	32	27	17	9	
B. subtilis	6633	0.5	M	UC	142	122	91	56	11	7	-
		1	UC	UC	120	103	77	44	8	1	
		1.5	UC	UC	101	97	69	30	4	0	
S. abony	6017	0.5	M	UC	123	115	96	41	22	1	-
		1	UC	UC	112	106	83	39	17	4	
		1.5	UC	UC	101	94	75	21`	10	0	

Table no 11: Result after incubation at 25°c - 30°c for 120 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
C. albicans	10231	0.5	UC	UC	96	63	52	36	18	7	-
		1	UC	UC	85	49	33	21	11	3	
		1.5	UC	UC	71	34	28	19	7	0	

Table no 12: Result after incubation at 25°c - 30°c for 168 Hrs.

Micro-organism	ATCC No.	Conc %v/v	Dilutions								Log reduction/ contact time (Min.)
			10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
A.brasiliensis	16404	0.5	UC	UC	122	100	85	44	25	12	-
		1	UC	UC	107	98	74	38	19	5	
		1.5	UC	UC	93	87	62	26	8	0	

Here the results shows that the growth of organisms in not inhibited at any concentration for a period of 5 mins hence the concentration is selected to be 1% and the time required is maintained at 10 mins.

SWAB ANALYSIS[1,4]

Choose a standard area to swab (minimum of 10cm^2). Record the standard area.

1. Clean, powder-free gloves must be worn.

2. Pre-mark the standard area on the surface to be wiped.
3. Wet each swab with appropriate solvent.
4. Wipe the swab across the pre-marked surface from left to right using an even pressure and holding the swab flat against the surface.
5. Continue until the whole surface has been wiped.
6. Re-wipe again top to bottom.
7. Re-wipe again bottom left to top right.
8. Re-wipe again top left to bottom right.
9. Used swabs should be sealed in labelled sampling containers appropriate for the storage of the analytes of interest. Record the sample name, site, date and time and sampler in a notebook or equivalent.
10. If a template has been used, it must be cleaned appropriately before using it at another site.
11. A field blank should be taken by wetting the swab/s with the solvent and placing the swab in the jar.

Table no 13: Swab analysis result of TOTASEP.

Sr.no	Micro-organism	Dilution	Results
1	*E. coli* (4157)	10^4	No Growth
2	*S. aureus* (6538P)	10^3	No Growth
3	*P. aeruginosa* (15442)	10^3	No Growth
4	*B. subtilis*(6633)	10^3	No Growth
5	*S.abony(6017)*	10^3	No Growth
6	*C. albicans*(10231)	10^3	No Growth
7	*A. brasiliensis*(16404)	10^3	No Growth

AGAR WELL DIFFUSION METHOD[12]

Procedure

- Agar plates are prepared and the specified concentration of micro-organisms to be challenged are spread over the surface
- The wells are prepared by using the sterile cork borer or a tip.
- A volume (20–100 mL) of the antimicrobial agent at desired concentration is introduced into the well
- Incubate the plates for 24hr. the antimicrobial agents diffuses through the agar medium and zone of inhibition is formed. Measure the zone of inhibition

Table no 14: Zone of inhibition of TOTASEP by agar well diffusion method.

Sr. No	Concentration of the disinfectant(%V/V)	Zone of inhibition (mm)
1	0	0
2	1	5
3	2	8
4	3	15
5	4	18
6	5	25

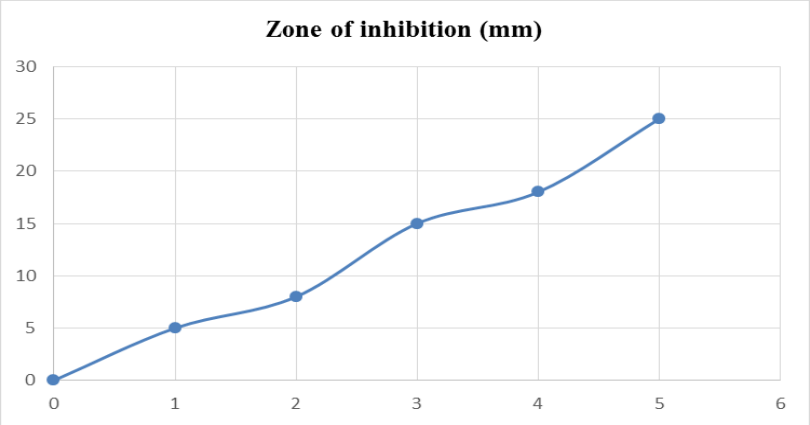

Fig no 4: Graph of Zone of Inhibition.

The disinfectant (TOTASEP) gives more than 7 log reduction for all the above mentioned micro-organism in 10 min.

Table No. 15: Calculation of the log reduction values on all microbes.

Micro-Organisms (ATCC No. / NCIM No.)	Culture Dilution [Viable count]	Contact time (Min.)	10	20	30	Conclusion
E. coli	A= 7.72 x 10^7 log A= 7.887	CFU / 0.1 mL	0	0	0	Totasep 1% solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.887	7.887	7.887	
S. aureus	A= 7.30 x 10^7 log A= 7.863	CFU / 0.1 mL	0	0	0	Totasep 1%solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.863	7.863	7.863	
S. abony	A= 9.24 x 10^7 log A= 7.965	CFU / 0.1 mL	0	0	0	Totasep 1%solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.965	7.965	7.965	
B. subtilis	A= 8.65 x 10^7 log A= 7.937	CFU / 0.1 mL	0	0	0	Totasep 1%solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.937	7.937	7.937	

P. aeruginosa	A= 8.68 x 10^7 log A= 7.938	CFU / 0.1 mL	0	0	0	Totasep 1%solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.938	7.938	7.938	
A.brasiliensis	A= 7.93 x 10^7 log A= 7.899	CFU / 0.1 mL	0	0	0	Totasep 1%solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.899	7.899	7.899	
C. albicans	A= 9.62 x 10^7 log A= 7.983	CFU / 0.1 mL	0	0	0	Totasep 1%solution gives >7 Log Reduction in 10 Mins
		Log of CFU (B)	0	0	0	
		Log reduction (A-B)	7.983	7.983	7.983	

CONCLUSION

Initially the viable count was determined to identify the log reduction capacity of the standard microbial culture maintained in the organism, followed by the investigation of the viable count, the disinfectant TOTASEP at a concentration 1% v/v demonstrated more than 7 log reduction when investigated by membrane filtration and direct inoculation method. After performing the study, 7 log reduction swab analysis was done. The disinfectant TOTASEP having concentration of 1% v/v was found to be effective against the standard bacteria on various surfaces, subsequently the activity of the disinfectant was supervised by agar diffusion method that demonstrated if the concentration of disinfectant increases the zone of inhibition also increases. Therefore it is concluded that after performing several test the results of the investigated demonstrated that the disinfectant was highly effective and can be applied for the use for different purposes at the sterile manufacturing facility during spillage and any other hazardous situations. Therefore the validation of the incoming disinfectant was performed as well as the efficacy was tested which states that the disinfectant TOTASEP was found to be effective for the specified purpose.

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REFERENCES

1. Jadhav R Raut G. Human journals efficacy of some antiseptics and disinfectants; A review. Int J of Pharm & Pharm Res (IJPR), 2015; 4(4): 182-197.
2. Ribeiro MM. Efficacy and effectiveness of Alcohol in the disinfection of Semi- critical materials: A systemic Review. Rev Lat-Am-enferm, 2015; 23(4): 741-52.
3. Salan fathy Ahmed Abd El Al. Determination of the bactericidal activity of chemical disinfectants against bacteria according to the DVG –Guidelines. Hyg Med, 2008; 33(1): 463-71.
4. Edward RW Haffmann RK .Microbiological contamination control; Prepared by: The Biological Contaminatioin Control Committee of the American association for Contamination Control; Oct 13 1966.
5. Ughlig S Simon K. Validation of efficacy methods for antimicrobials used on hard surface. Quo data, Draft Report, 2009-11-20.
6. Dr Raval K.A review on Cleaning validation sampling techniques. EJPMR, 2016; 3(7): 202-206.
7. Balouiri M. Methods for in vitro evaluating antimicrobial Activity: A review. J of Pharm Anal (ELSEVEIR), 2016; 6: 71-79.
8. Rogers M .Disinfection Efficacy testing for Critical environments. Steris Life Sciences; PDA SE Chapter; Greenville, NC, April 18 2013.
9. Nema S Ludwig J. Pharmaceutical Dosage Forms – Facility Design. Sterilization and Processing Parentral medications; Infrm Healthcare Publications; Third edition, 2: 241-256.
10. Cooper and Gunn; Tutorial Pharmacy; CBS Publishers and Distributors; 6th Edition, 353-366.
11. Linda A. Felton; Remington Essentials of Pharmaceutics; Pharmaceutical Press Publication; 1st Edition, 500-515.
12. Richard A. Harvey, Pamela Champ; Lippincott's Illustrated Reviews; The Point Publication; 4th Edition, 319-336.
13. Kevin. L. Williams; Microbial Contamination Control in Parentral Manufacturing, Drugs and Pharmaceutical Sciences; Marcel and Dekker Inc., 20-350.
14. Dr. kokare Chandrakant; Pharmaceutical Microbiology Principles and Applications; Nirali Prakashan, 14.1-14.30.
15. Avis. E. Kenneth; A. Herbert; Lachmann leon and libermann; Pharmaceutical Dosage Forms Parenteral Medications; Second edition, 1: 222-370.