



SELECTION AND MICROBIAL CONTROL OF DISINFECTANTS AND VALIDATION OF DISINFECTANT MICROBIAL EFFICACY IN THE PHARMA & BIOPHARMACEUTICAL INDUSTRY: A CASE STUDY

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Abstract: We have evaluated the efficacy of different neutralizing solutions for use in disinfectant efficacy assays. Our approach used for comparisons between the recoveries of low inocula in different treatment populations. The challenge organisms employed were those described by the Bacilloid special for use in determining germicidal, bactericidal, sporicidal, and fungicidal activity of disinfectants. The manufacturing processes are carried out in controlled environment for which we have classified areas. The microbial load in the classified area must be controlled to ensure asepsis. For this classified areas are regularly disinfected to ensure that the microbial loads are kept under control. The disinfectants are used for sanitization of floors, walls, ceilings, surfaces and other articles in the classified areas of laboratories. It is important to check the disinfectant efficacy before it is used in our facility. This is checked by various methods. The disinfectant used at facility across the sites is Bacilloid special 0.5% v/v for routine monitoring and 2.0% v/v for fumigation purpose. The Bacilloid special contains: 1, 6 Dihydroxy, 2-5 Dioxahexane - 11.2g, Glutaraldehyde - 5.0g, Benzalkonium chloride - 5.0g and Alkyl urea derivatives - 3.0g. Bacilloid special is a disinfectant that is specially designed for the disinfection of high risk areas. It has got excellent cleansing properties, a broad spectrum germicidal action with corrosion inhibitors and cleanser hence this is selected for disinfection. The study was conducted at one location of facility, but the environmental isolates of all facilities were used as a challenged organisms hence the study outcome are applicable for facilities.

Keywords: Bacilloid, Microbial Control, efficacy

INTRODUCTION

We evaluated the efficacy of different neutralizing solutions for use in disinfectant efficacy assays. Our approach used comparisons between the recoveries of low inocula in different treatment populations. The challenge organisms employed were those described by the Bacilloid special for use in determining germicidal, bactericidal, sporicidal, and fungicidal activity of disinfectants.

The manufacturing processes are carried out in controlled environment for which we have classified areas. The microbial load in the classified area must be controlled to ensure asepsis. For this classified areas are regularly disinfected to ensure that the microbial loads are kept under control.

The disinfectants are used for sanitization of floors, walls, ceilings, surfaces and other articles in the classified areas of laboratories. It is important to check the disinfectant efficacy before it is used in facility. This is checked by various methods. The disinfectant used at facility is Bacilloid special 0.5% v/v for routine monitoring and 2.0% v/v for fumigation purpose.

The Bacilloid special contains: 1, 6 Dihydroxy, 2-5 Dioxahexane-11.2g, Glutaraldehyde-5.0g, Benzalkonium chloride-5.0g and Alkyl urea derivatives-3.0g. Bacilloid special is a disinfectant that is specially designed for the disinfection of high risk areas. It has

got excellent cleansing properties, a broad spectrum germicidal action with corrosion inhibitors and cleanser hence this is selected for disinfection.

The study was conducted at one location of facility, but the environmental isolates of all facilities were used as a challenged organisms hence the study outcome are applicable for facilities. Our objective is to verify the efficacy of disinfectant used in the controlled areas, in reducing the microbial load.

Abbreviations

- CFU – Colony forming unit.
- SCDM – Soybean casein digest medium.
- MIC – Minimum inhibitory concentration.
- NCIM – National collection of industrial microorganisms.

Material and Equipment's

- Disinfectant solution
- Neutralizing solution
- Media with neutralizant
- soya bean casein digest agar.
- Sarbourauds dextrose agar.
- Normal saline
- Saline with tween 80
- Sterile glassware
- sterile membrane filtration assembly
- Filtration flask

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- 0.22 membrane filter
- sterile swab.
- Autoclave
- incubator 20 – 25 C
- Incubator 30 – 35 C
- Biosafety cabinet.

Microorganisms used in the study

- *Pseudomonas aeruginosa* NCIM No.2200
- *Candida albicans* NCIM No 3471
- *Staphylococcus aureus* NCIM No 2097
- *Bacillus subtilis* NCIM No 2063
- *Aspergillus niger* NCIM No 1196

Efficacy of Bacilloid special 0.5% (V/V) solution

The study was conducted on selected standards microbial strains as well as environmental isolates. The test microorganisms were kept in bacilloid special 0.5% (V/V) solution for contact time of 3 min, 5 min, 10 min, 15 min and 20 min. after this contact time, the whole preparation was filtered through the sterile membrane filter and the membrane filter was rinsed with neutralizing solution 1 (already identified previously to be effective) for the proper neutralization of the inhibitory effects of the disinfectant used. After neutralization filter was placed on the soybean casein digest agar plates. The plates then were incubated at the appropriate temperature and time. The plates after incubation were observed for colony forming units and long reduction was calculated.

Results and conclusion of phase 1 study

For contact time of 3 minutes the log reduction of 5.54 was achieved in case of *Aspergillus niger* (standard microorganism) which meets the acceptable criteria of minimum 5 log reduction.

For contact time of 5 minutes the log reduction of 6.33 was achieved in case of *Aspergillus niger* (standard microorganism) which meets the acceptable criteria of minimum 5 log reduction.

For contact time of 10 minutes the log reduction of 6.95 was achieved in case of *Pseudomonas aeruginosa* (standard microorganism) which meets the acceptable criteria of 5 log reduction.

For contact time of 15 and 20 minutes the log reduction of 8.54 was achieved in case of *Candida albicans* (standard microorganism), *Bacillus spp.* Which meets the acceptable criteria of minimum 5 log reduction.

Log reduction in the presence of disinfectant 0.5% with different contact time.

Name of organism	Log reduction				
	3 min	5 min	10 min	15 min	20 min
<i>Candida albicans</i>	5.57	6.73	7.21	8.54	8.54
<i>Aspergillus niger</i>	5.54	6.33	7.37	8.51	8.51
<i>Pseudomonas aeruginosa</i>	5.8	6.56	6.95	8.61	8.61
<i>Bacillus subtilis</i>	5.88	6.87	7.41	8.62	8.62
<i>Staphylococcus aureus</i>	6.78	7.19	7.78	8.85	8.85
<i>Micrococcus spp.</i>	5.82	6.88	7.41	8.65	8.65

As per the study the 3 minutes contact time is sufficient to control the microbial load as 5 log reduction was achieved in 3 minutes. Hence the contact time of 10 minutes currently used for the sterilization of classified area is sufficient to control the buildup of microbial load.

Phase 2: Surface challenge test

in the study environmental isolates as well as standard strains of various microorganisms were spiked on the working surface of biosafety cabinet in 25cm² area enclosed in the stainless stencil, after that, the area was disinfected by the bacilloid special 0.5% (v/v) solution and was allowed for contact time of 10 min. the spiked area then was wiped by the sterile swab. After sampling, the swab was rinsed in sterilized neutralizing solution-1 and the content was pour plated having soya bean casein digest agar with neutralizant-1. The plates were incubated for the recovery of spiked organisms. For positive control the standard strains of various microorganisms were spiked on the working surface of biosafety cabinet in 25 cm² area enclosed in the stainless stencil, this area was not disinfected. The log reduction in the population of microorganisms was recorded and the results were compared with recovered spiked microorganisms.

For 10 minutes contact time the minimum 6.50 log reduction and maximum of 7.60 log reduction was achieved.

Final conclusion for bacilloid special 0.5% (v/v) solution

on the basis of results obtained and the analysis carried out it is clearly evident that always for all the environmental isolates of facility, used in the study, the acceptance criteria of at least 5 log reduction of microorganisms when kept in contact for 10 minutes with bacilloid special 0.5% (v/v) solution has been met. This is true when the test organisms are in contact with bacilloid special 0.5%(v/v) solution for 10 minutes in test tube (phase 1 of the study) and also when the contact is allowed on the biosafety cabinet surface (phase 2 of the study). It was observed that in all the cases all the organisms having concentration as high as

10^8 cfu/ml showed the minimum of 6.50 log reduction and maximum of 7.60 log reduction when kept in contact for 10 minutes. Hence bacillocid special 0.5% (v/v) solution which is used as a sanitization agent is effective for all facilities.

Efficacy of bacillocid special 1.0% (v/v) solution

Phase 1: Establishing the recovery of spiked organisms by membrane filtration method:

The study was conducted on selected standards microbial strains as well as environmental isolates. The test microorganisms were kept in bacillocid special 1.0% (v/v) solution for contact time of 3 min, 5 min, 10 min, 15 min, and 20 min. after this contact time, the whole preparation was filtered through the sterile membrane filter and the membrane filter was rinsed with the neutralizing solution 1 (already identified previously to be effective) for the proper neutralization of the inhibitory effects of the disinfectant used. After neutralization filter was placed on the soybean casein digest agar plates. The plates then were incubated at the appropriate temperature and time. The plates after incubation were observed for colony forming units and log reduction was calculated.

Conclusion of phase 1 study

For contact time of 3 minutes the log reduction of 6.29 was achieved in case of staphylococcus spp (environmental isolate) which meets the acceptance criteria of minimum 5 log reduction. For contact time of 5 minutes the log reduction of 6.80 was achieved in case of *Pseudomonas aeruginosa* (standard microorganism) which meets the acceptable criteria of minimum 5 log reduction.

For contact time of 10 minutes, 15 and 20 minutes the log reduction of 8.54 was achieved in case of *Candida albicans* (standard microorganism), bacillus spp. (environmental isolate) which meets the acceptable criteria of minimum 5 log reduction.

Log reduction in the presence of disinfectant 1.0% with different contact time.

Name of organism	Log reduction				
	3 min	5 min	10 min	15 min	20 min
<i>Candida albicans</i>	6.56	6.98	8.54	8.54	8.54
<i>Aspergillus niger</i>	6.41	6.84	8.51	8.51	8.51
<i>Pseudomonas aeruginosa</i>	6.41	6.8	8.61	8.61	8.61
<i>Bacillus subtilis</i>	6.6	6.97	8.62	8.62	8.62
<i>Staphylococcus aureus</i>	6.98	7.5	8.85	8.85	8.85
<i>Micrococcus spp.</i>	7.1	7.79	8.65	8.65	8.65

As per the study the 3 minutes contact time is sufficient to control the microbial load, as 5 log

reduction was achieved in 3 minutes. Hence the contact time of 3 minutes is used for surface challenge study.

Phase 2: Surface challenge test

In the study environmental isolations as well as standard strains of various microorganisms were spiked on the working surface of biosafety cabinet in 25 cm² area enclosed in the stainless steel stencil after that, the area was disinfected by the bacillocid special 1.0% (v/v) solution and was allowed for contact time of 10 min. the spiked area then was wiped by the sterile swab. After sampling, the swab was rinsed in sterilized neutralizing solution – 1. The plates were incubated for the recovery of the spiked organisms. The log reduction in the population of microorganisms was recovered. For 3 minutes contact time the minimum 7.09 log reduction and maximum of 8.01 log reduction was achieved.

Final conclusion for bacillocid special 1.0% (v/v) solution

on the basis of results obtained and the analysis carried out it is clearly evident that always for all the environmental isolates used in the study, the acceptance criteria of at least 5 log reduction of microorganisms when kept in contact for 3 minutes with bacillocid special 1.0% (v/v) solution has been met. This is true when the test organisms are in contact with bacillocid special 1.0% (v/v) solution for 3 minutes in the test tube (phase 1 of the study) and also when the contact is allowed on the biosafety cabinet shelf (phase 2 of the study). It was observed that in all the cases all the microorganisms having concentration as high as 10^8 cfu / ml showed the minimum of 7.09 log reduction and maximum of 8.01 log reduction when kept in contact for 3 minutes.

Efficacy of Bacillocid special 2.0% (v/v) solution

Phase 1: Establishing the recovery of spiked organisms by membrane filtration method

The study was conducted on selected standards microbial strains as well as environmental isolates. The test microorganisms were kept in bacillocid special 2.0% (v/v) solution for contact time of 3 min, 5 min, 10 min, 15 min and 20 min. after this contact time, the whole preparation was filtered through the sterile membrane filter and the membrane filter was rinsed with the neutralization solution 1 (already identified previously to be effective) for the proper neutralization of the inhibitory effects of the disinfectant used. After neutralization filter was placed on the soybean casein digest agar plates. The plates then were inhibited at the appropriate forming units and log reduction was calculated.

Results and conclusion of phase 1 study:

For contact time of 3 minutes the log reduction of 7.41 was achieved in case of *Aspergillus niger* (standard microorganism) which meets the acceptable criteria of minimum 5 log reduction.

For contact time of 5 minutes, 10 minutes, 15 minutes and 20 minutes the log reduction of 8.54 was achieved in case of *Candida albicans* (standard microorganism), *Bacillus* spp. (environmental isolates) which meets the acceptance criteria of minimum 5 log reduction.

Log reduction in the presence of disinfectant 2.0% with different contact time.

Name of organism	Log reduction				
	3 min	5 min	10 min	15 min	20 min
<i>Candida albicans</i>	7.45	8.54	8.54	8.54	8.54
<i>Aspergillus niger</i>	7.41	8.51	8.51	8.51	8.51
<i>Pseudomonas aeruginosa</i>	7.37	8.61	8.61	8.61	8.61
<i>Bacillus subtilis</i>	7.9	8.62	8.62	8.62	8.62
<i>Staphylococcus aureus</i>	8.32	8.85	8.85	8.85	8.85
<i>Micrococcus</i> spp.	8.65	8.65	8.65	8.65	8.65

As per the study the 3 minutes contact time is sufficient to control the microbial load, as 5 log reduction was achieved in 3 minutes. Hence the contact time of 3 minutes is used for surface challenge study.

Phase 2: Surface challenge test

In the study environmental isolates as well as standard strains of various microorganisms were spiked on the working surface of biosafety cabinet in 25 cm² area enclosed in the stainless steel stencil. After that, the area was disinfected by the bacillocid special 2.0% (v/v) solution and was allowed for contact time of

10 min. the spiked area then was wiped by the sterile swab. After sampling, the swab was rinsed in sterilized neutralizing solution-1 and the content was pour plated having soybean digest agar with neutralizant-1. The plates were incubated for the recovery of spiked organisms. The log reduction in the population of microorganisms was recorded. For 3 minutes contact time the minimum 8.06 log reduction and maximum of 8.97 log reduction was achieved.

Final conclusion for bacillocid special 2.0% (v/v) solution

On the basis of results obtained and the analysis carried out it is clearly evident that always for all the environmental isolates. The acceptance criteria of at least 5 log reduction of microorganisms when kept in contact for 10 minutes with bacillocid special 2.0% (v/v) solution has been met. This is true when the test organisms are in contact with bacillocid special 2.0% (v/v) solution for 3 minutes in the test tube (phase 1 of the study) and also when the contact is allowed on the biosafety cabinet shelf (phase 2 of the study). It was observed that in all the cases all the organisms having concentration as high as 10⁸ cfu/ml showed the minimum of 7.09 log reduction and maximum of 8.01 log reduction when kept in contact for 3 minutes. Hence bacillocid special 2.0% (v/v) solution which is used as an agent is effective for all facilities of manufacturing facility.

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