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Comparative Study between Quaternary Ammonium Compounds and Formalin in Clean Room Sanitation

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Abstract: *Sanitization of Pharmaceutical clean room is always been a burning issue for clean room experts. This is not only because of the sensitivity of product manufactured in clean room but also due to emergence of natural resistance of Micro-organisms , regulatory requirement ,which are becoming strict day by day and the effect of chemicals used for the disinfection of clean on human health. Today there is a very vast range of chemicals available for clean room sanitation and each of them has its own disadvantages and advantages. Formaldehyde and Quaternary ammonium compound are being used for clean room sanitation. A comparative study was conducted between these two compounds to find out advantages and disadvantages of these two chemicals. For this purpose a clean room facility was selected which was classified as "Grade B" and Grade "C". These Grades are the filtration grades classified by World Health Organization for the aseptic processing of Parenteral products. Grade B is very high risk area as the operator standing in this grade throughout the batch processing and doing all activities. According to the USP, the biggest source of microbial contamination is a man. A simulation batch processing activity was carried out and then the area was de-contaminated with Quaternary ammonium compound. Samples were collected after area decontamination. Again another simulation batch processing activity was performed and this time area was decontaminated with Formalin (Formaldehyde). Samples were collected after decontamination and the results of both (Formaldehyde and Quaternary ammonium compound) were compared.The results shows that the Quaternary ammonium compound are much better than formaldehyde and have less health effect on human.*

Keywords: *Formalin, Quaternary Ammonium Compound (QUATS), CFU, Sanitization, Clean room*

I. Introduction

The right selection of chemicals for clean room sanitization is always been a challenge. The nature of the process, the microbial flora and the risk associated with the use of chemical is debatable. Formalin has been used for clean room sanitation. Now there are some new compounds like Quaternary ammonium compounds (Biocide X and Vanoquat), which are being introduced in clean room sanitation.There is no comprehensive study was conducted to compare the effectiveness of Formalin and Quaternary ammonium compounds. Further all studies were conducted on lab scale where many factors that can affect the effectiveness of chemicals were not considered. Comparative study between QUATs and Formalin was performed in clean room. Worst case locations were selected on the basis of work load, number of person in area and the time duration of operation. Microbial effectiveness of both compounds. Microbial environmental monitoring including exposure of TSA plates, Swab test and recovery time after sanitization of area. Microbial test are recovery test and run in duplicate to achieve assurance level Parenteral product processing require very sophisticated techniques and the running cost of the process is very high. Further if product is failed in testing then there is no chance of recovery and the batch is rejected.The approach described here is widely accepted at international level to determine the effectiveness of antimicrobial chemical agents.

II. Material and Method

1.1 Instrument & Equipment:

Steam sterilizer 800L from Buxton model no. 9502, USA was used for the sterilization of uniforms and accessories. Microbiological air quality sampler from Merck, Germany was used for environmental monitoring. Master Fogger from Tri-jet Fogmaster, USA was used for Sanitation of Grade B and Grade C facility of Getz Pharma (Pvt) ltd. Systech Steam Sterilizer from Switzerland was used for the sterilization of media (Tryptic Soya Agar- TSA & Tryptic Soya Broth- TSB), Petri plates and Test tubes. All sterile TSA plates and sterile TSB tubes were pre-incubated for 72hrs. in order to make sure that media in plates and tubes is not contaminated. This study was performed in sterile facility of Getz Pharma where Liquid Vial filling line, Powder Vial Filling

line from Petals Innovative private Limited, India and Liquid Ampoule filling machine from Cozzoli, USA are installed**.**

1.2 Reagents and accessories:

Biocide X (1:100), Vanoquat (1:50), Formalin 37%, Potassium Permanganate, Sterile uniforms for grade B area and non-sterile uniform for grade C, Sterile lint free duster, Sterile Spray bottles, Sterile TSA plates, Sterile TSB tubes, Sterile Surgical gloves- Kimtech pure, Filtration Assemblies 293mm Millipore, Pre-filter 293mm Millipore, Sterile Swabs, Filter 0.22micro-meter 293mm Millipore, Sterile Isopropyl Alcohol 70%, Sterile Wipe, Sterile wringer trolley, Face Mask, SS plates & Respirator

1.3 Sample Preparation:

Sterile TSA plates were prepared by pouring sterile TSA into glass petri plates of 90mm. These plates were incubated at 32.5±2.5ºC for 72 hours and then used. Similarly Sterile TSB was poured in 100ml sterile test tube and incubated for 72hours. After 72 hours these tubes were used. Critical locations in Grade B and Grade area were identified (See table 1 & 2) on the basis of risk of microbial contamination.Environmental air samples were taken from different location during operation condition through Microbiological air samples and TSA plates. Critical locations with respect to microbial contamination were sampled through sterile swab for each location and that swab were inoculated in Sterile TSB test tube.

1.4 Sanitation procedure

After completion of work collect all ampoules / vials or their pieces, rubber stoppers, flip off seals and other materials through duster, which fall on machine or SS table. Collect all material through scraper which falls on floor during manufacturing, filtration, filling, and sealing process and transfer in waste bin. Take lint free cloth or mop soaked in cleaning and sanitizing agent (sterile lint free cloth or sterile mop in case of grade A and B) or spray the cleaning and disinfectant agent on to the surface to be cleaned and disinfected (sterile cleaning and disinfectant agents for grade A & B).Wipe to clean and disinfect all the surfaces and items. Fog the area with Sanitizing agent as per rotation policy (In this case we performed three weeks study and for every week, we selected one sanitizing agent (Biocide X) out of three. Second week we used Vanoquat and third week we used Formalin. As per standard operating procedure, the fogging frequency was followed, which was on every one alternate day in a week.

III. Observation and Results

In our study we selected two different classes of chemicals used in clean room sanitation. These chemicals are not only using in Pharmaceutical clean room but also in operation theatre, in sterile compounding section of hospital pharmacy and veterinary medicine manufacturing areas. TSA and TSB are the simplest medium and almost all kind of microorganism can grow on these medium. Colonial characterization of Microorganisms is very simple on TSA media. To further conform the suitability of media. It is GMP practice to run Growth promotion test on media to check whether media is compatible with environmental flora or not. This is known as GPT test.Conventional method of microbial evaluation such as the environmental monitoring with sterile TSA plates and sterile TSB swabs. Microbial recovery method are very sensitive and preferably run in duplicate to evaluate the recovery of the method. The results are always reported on average basis. Sampling locations were selected on the basis of high risk to microbial contamination.

Table 3
Sampling Locations of Environmental Monitoring in Grade B
(TSA Plates & Swab sample)

Location of Fogging		Location in room	No. of samples / location*	Sampled by
Room No.	Filtration Class			
301	B	Filling Machine, Curtains, wall, window ceiling & Floor	2	QC Microbiologist
301	B	Filling Machine, Curtains, window, wall, ceiling & Floor	2	
302	B	Cabinet, wall, ceiling & Floor	2	
303	B	Curtains, wall, ceiling & Floor	2	
304	B	Wall, ceiling, window & Floor	2	
305	B	Filling Machine, Curtains, wall, ceiling & Floor	2	
306	B	Wall, ceiling & Floor	2	
307	B	Wall, ceiling & Floor	2	
308	B	Wall, ceiling & Floor	2	
309	B	Wall, ceiling & Floor	2	
310	B	Wall, ceiling & Floor	2	
311	B	Wall, ceiling & Floor	2	
312	B	Wall, ceiling & Floor	2	
313	B	Wall, ceiling & Floor	2	

*Duplicate samples were taken from each location.

Table 4
Sampling Locations of Environmental Monitoring in Grade C
(TSA Plates & Swab samples)

Location of Fogging		Sampling location in room	No. of samples/location*	Sampled by
Room No.	Filtration Class			
101	C	Wall, window ceiling & floor	2	QC Microbiologist
102	C	Vial washing machine, wall, window ceiling & floor	2	
103	C	Cabinet, wall, ceiling & floor	2	
104	C	Cabinet , wall, ceiling & floor	2	
105	C	Wall, ceiling & floor	2	
106	C	wall, ceiling & floor	2	
107	C	Wall, ceiling & Floor	2	
108	C	Wall, ceiling & Floor	2	
109	C	Wall, ceiling & Floor	2	
401	C	Wall, ceiling & Floor	2	

*Duplicate samples were taken from each location.

Results showing in table 5 & table 6 are indicating that Quaternary ammonium compounds (Biocide X and Vanoquat) are much better than Formalin in clean room sanitation. There are some other reasons behind the effectiveness of QUATs and Formalin Handling of Formalin is very risky as the person need to prepare himself very well. Further air in the area should be stagnant and it remain stagnant for 6 to 8 hours until the formalin reached to all location. Further mixing of Formalin with Potassium Permanganate is produces black compound with a Formalin gas. Formalin cannot be filtered as it is mandatory requirement that chemical agents use for sanitization of clean room should be sterile itself. On the other hand there are no such issues of using QUATs as they can be sterile filter. They can be diluted with water, so a very low concentration is required to sanitize the area. Their MSDS are available and all safety requirement are properly addressed. There is no need to switch off air handling unit of area and the sanitization can be done in dynamic condition. Comparatively less quantity is used for clean room sanitation.Further there is natural tendency that Microorganisms develop resistance against disinfectant agent if there is no rotation policy follow. In case of QUATs, there are many compounds which can be used on rotation basis.

Table 5 – Results of Environmental monitoring and Swab sampling of Grade C area

Room No.	Observation and Results – TSA Plates* (No. of CFU)		Observation and Results - TSB Swab* (No. of CFU)		Acceptance criteria
	Vanoquat	Formalin	Vanoquat	Formalin	
101	15	22	11	23	Alert limit: 25CFU Action limit: 50CFU
102	14	25	18	21	
103	11	28	14	29	
104	19	35	14	26	
105	16	18	11	18	
106	09	29	09	33	
107	21	39	15	31	
108	18	27	18	27	
109	15	11	13	16	
401	16	55	11	55	

*Average results were reported

Table 6 – Results of Environmental monitoring and Swab sampling of Grade B area

Room No.	Observation and Results – TSA Plates* (No. of CFU)			Observation and Results -TSB Swab* (No. of CFU)			Acceptance criteria
	Vanoquat	Biocide X	Formalin	Vanoquat	Biocide X	Formalin	
201	02	01	03	01	00	03	Alert limit: 03CFU Action limit: 05CFU
301	01	02	02	02	01	03	
302	01	01	06	01	02	05	
303	01	01	04	01	01	04	
304	02	01	05	01	02	05	
305	02	00	02	02	01	06	
306	01	01	03	01	00	03	
307	02	02	04	02	01	04	
308	01	01	05	01	02	05	
309	01	00	05	02	02	04	
310	01	01	03	01	01	03	
311	02	01	04	01	02	04	
312	02	02	04	02	00	03	
313	02	01	05	02	01	05	

*Average results were reported

IV. Conclusion

The comparative study between QUATs and Formalin reveals that microbial control will be compromised with the use of Formalin but not in the case of QUATs. Swab samples taken from different location were within limit in case of QUATs. The CFU reported here in case of QUATs are the CFU observed mostly in floor samples which is considerably the most dirties location as compared to the other locations. The swab results of Formalin were also alarming as not only a high count observed on floor but also on walls, cabinet and machine as well which are the critical locations as the product may come in contact with machine parts. Since the product is considered to be sterile so the product contact surfaces must be sterile. As per USP there are some limitation that we have to pick some samples for sterility testing as this test is a destructive test. So this is a statistical test. Therefore as a pharma manufacturer, product contact surfaces must be given a high priority. Further we also observed an increased pattern of CFU in Formalin which is an indication of poor response against Formalin, emergence of resistance against Formalin. In case of QUATs since there is no risk to operator, Operator vigilantly monitor all areas during sanitization and ensure that all areas are covered.

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