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Clean Room Start Up: Evaluation of a Combined Sanitization Regimen Using Ready-To-Use (RTU) Quaternary Ammonium and RTU Hydrogen Peroxide/Peracetic Acid

A clinical supply manufacturer was adding a new integrated filling operation to its aseptic filling capabilities. The new pilot lab area provides a Grade C background environment with adjacent Grade D transition airlocks for material and personnel.

The traditional sanitization/disinfection used by the site included the routine use of hydrogen peroxide/peracetic acid ready to use (RTU), 70% isopropyl alcohol (IPA), and hot Water for Injection (WFI).

The site explored disinfection products currently available and utilized at the adjacent affiliated commercial sites after realizing the limited cleaning effectiveness of the established regimen. As the site strategized to determine the revised regimen, the need for a global approach was considered to synergize all related activities.

The site reviewed several cleaning and disinfection formulations currently available in the United States (US) as well as globally. Not all disinfectants and sporicides are available globally and, thus, the choices were limited. In addition to global availability, the site needed a formulation that met the following criteria:

- Demonstrated efficacy against vegetative bacteria, molds, and bacterial spores meeting cleanroom classifications for ISO-14644
- Chemically compatible with hydrogen peroxide/peracetic acid RTU
- Suitable for hard, non-porous surfaces common in cleanrooms
- Ready to use formulations
- Capable of handling multiple application methods
- With minimal residue after application visually
- Without a phenolic active ingredient

The disinfectant and sporicide were chosen by the site and a review of all efficacy data provided by the disinfectant manufacturer which also included the coupon testing representing multiple cleanroom surfaces which are representative of the cleanroom site construction. The Disinfectant Efficacy Testing (DET) studies performed passed all acceptance criteria and were accepted by the site to support the initial efficacy evaluation. Because of the design and results of the solution manufacturer DET studies, an in-house DET study was not performed before the execution of the Environmental Monitoring Performance Qualification (EMPQ), which is also referred to as in situ testing. Furthermore, as this was a newly constructed area, there is no microbiological history to evaluate for an environmental isolate testing. An objective of this functional testing was to verify that the solution manufacturer DET results, coupled with the newly implemented sanitization process, provide a robust system for microbiological organism reduction to comply with Grade C/D classifications. The site would leverage the efficacy data available through the solution manufacturer and rely on empirical data collected during routine environmental monitoring (EM).

Real-time EM data collection and data trending must support the routine use of sanitization solutions. Typical organisms isolated represent the actual flora recovered, and solution efficacy is directly linked to this worst-case performance in a Grade C environment. Site bioburden limits for Grade C areas allow for measurable growth (percent growth as well as magnitude) as compared to the zero limit in Grade A areas. This limit enables recovered microbial counts to directly measure/calculate the expected solution efficacy versus actual amounts delivered.

The validation study links empirical data generated to establish and verify solution efficacy (in situ) with real application in controlled areas.

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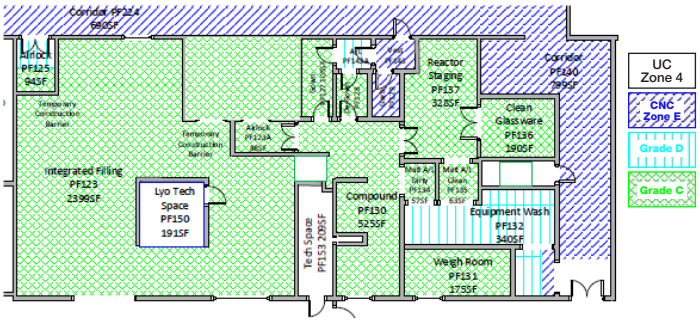
Figure 1 : Facility site layout



Evaluation Protocol

The site evaluation of quaternary ammonium RTU focused on efficacy as determined through direct application following site construction. Phase I construction activities were completed, and the area was ready for the EMPQ.

Before beginning the EMPQ, the constructed area was cleaned of all debris and residual construction materials. Once the debris and materials were removed, the area was washed with hot WFI followed by baseline sampling of all air and surface sites in multiple phases. The baseline phases (i.e., T=0, T=1, and T=2) were intended to assess the change in bioburden levels following each product application. The recovered growth was characterized, including Gram staining, to understand the nature (i.e., fungal, spore-forming, non-spore forming) and changes between each baseline phase.

The time zero (T=0) testing followed the post-construction debris removal and hot WFI wash included (105) Replicate Organism Detection and Counting (RODAC) surface samples and (100) active air samples using Tryptic Soy Agar (TSA) as the test media. Once sampled, the area was disinfected with quaternary ammonium RTU using overlapping, top to bottom, clean to dirty spray and wiping patterns. Surface mopping included overlapping figure-8 stroke patterns using the double bucket (dirty/rinse buckets) system on floors. After a 15-minute drying time, the area was sampled using the same surface and air sites.

This sampling was identified as T=1. The final baseline surface and air sampling, T=2, followed the application of hydrogen peroxide/peracetic acid RTU using the same techniques used for the quaternary ammonium RTU.

The completion of the baseline sampling, T=0, T=1, and T=2, leads then to the (1X) static and (3X) dynamic sampling, as described in ISO-14644.

The site map above details the facility layout and includes the environmental classification as related to the cleanroom areas.

Results

Each surface and air EM sample plate using TSA media was incubated for a minimum of 2-4 days at 30-35 °C, followed by a minimum of 3-5 days at 20-25 °C. Once incubated, each sample was examined under controlled conditions to evaluate the presence of colony-forming units (CFU). The enumerated growth was evaluated against the acceptance criteria for the Grade C/D controlled area and any impact determined.

The EMPQ data collected from the areas cleaned and disinfected were used to establish the various regimen used for routine facility cleaning and disinfection. The procedural requirements for routine cleaning and disinfection are as follows:

- Weekly – Quaternary ammonium RTU disinfection, followed by WFI wash of the windows
- Monthly – Quaternary ammonium RTU disinfection, followed by hydrogen peroxide/peracetic acid RTU for sporicidal disinfection, followed by WFI wash of the windows
- Shutdown – Residue Removal Process – Hot WFI water wash, followed by 70% IPA wipe down

Note: The EMPQ test period was extended to a 7-day period to cover the weekly regimen. Surface and air samples were pulled following static and dynamic conditions. The controlled areas were not cleaned or disinfected following each condition simulation.

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The tables below summarize the air and surface data from the T=0, T=1, and T=2 sampling results as expressed in terms of total isolate characterization (Gram stain results), total organism recovery (bacteria and mold)/total samples performed and percent reduction from T=0, T=1, and T=2.

Baseline Sample Regimen Summary:

Table 1-1: Baseline Sample Regimen (T=0, T=1, and T=2)	
T=0	Sampled baseline cleaning
T=1	Sampled after quaternary ammonium RTU cleaning
T=2	Sampled after hydrogen peroxide/peracetic acid RTU application

The organisms recovered from the surface and air samples were characterized, and Gram-stained as summarized below:

Table 1-2: Gram Stain Results (T=0, T=1, and T=2)

Organism Type	T=0 Amount	Percent Recovered	T=1 Amount	Percent Recovered	T=2 Amount	Percent Recovered
Gram Positive Rod	503	64	108	77	33	39
Gram Positive Cocci	224	29	15	11	38	45
Gram Negative Rod	2	0	5	3	5	6
Gram Negative Cocci	0	0	0	0	1	1
Mold	49	6	11	8	8	9
Yeast	7	1	0	0	0	0
Total No. of IDs	785	N/A	139	N/A	85	N/A

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The data expressed in total growth and total number of CFUs per plate are summarized below:

Table 1-3: Total Samples and Growth Recovered Results (T=0, T=1, and T=2)

Room #	Total number of samples			Total number of CFUs			Number of CFUs/Plate		
	T=0	T=1	T=2	T=0	T=1	T=2	T=0	T=1	T=2
123	44	44	44	190	70	6	4.32	1.59	0.14
123-A	10	10	10	46	2	2	4.6	0.2	0.2
124	21	21	21	140	1	12	6.67	0.05	0.57
125	10	10	10	26	0	1	2.6	0	0.1
127	13	13	13	25	3	7	1.92	0.23	0.54
128	7	7	7	33	1	0	4.71	0.14	0
130	21	21	21	62	13	0	2.95	0.62	0
131	12	12	12	39	15	20	3.25	1.25	1.67
132	16	16	16	35	5	4	2.19	0.31	0.25
133	12	12	12	47	12	26	3.92	1.00	2.17
134	7	7	7	21	1	0	3.00	0.14	0
135	6	6	6	24	3	1	4.00	0.5	0.17
136	12	12	12	37	4	0	3.08	0.33	0
137	16	16	16	37	8	7	2.31	0.5	0.44
143-A	6	6	6	23	1	1	3.83	0.17	0.17
Overall	**213**	**213**	**213**	**785**	**139**	**87**	**3.69**	**0.65**	**0.41**

Table 1-4: Change in Growth as Related to Sampling Event (T=0, T=1, and T=2)

Method	Percent change from T=0 to T=1	Percent change from T=1 to T=2	Percent change (overall)
Surface	(-) 448.1	(-) 248.5	(-) 1810.8
Air	(-) 680	(+) 80*	(-) 56

* The percent change for air sampling is the result of increased room activity. Additional personnel performed sampling related activities donned in minimal garments commensurate with Grade C/D classification. The data is not reflective of solution or regimen effectivity but the result of activity within the Grade C/D areas.

Conclusion

The sanitization process achieved a reduction in total bioburden from T=0 to T=2 (reference Tables 1-1 through 1-4). Baseline sampling of environmental flora resulted in an expected organism type percent recovery throughout the sanitization process; T=0 reduced total bioburden, T=1 reduced total bioburden and Gram-positive cocci and T=2 reduced total bioburden and Gram-negative rods.

Final baseline environmental flora represented an expected make up, from highest to lowest, Gram-positive cocci, Gram-negative rods, and mold (reference Table 1-2). The reduction in bioburden at each baseline sampling (including expected morphology reduction and overall percentage make up) coupled with the solution manufacturer quaternary ammonium RTU (Ready to Use) disinfectant efficacy testing results concluded that the new sanitization process is effective, and provide a sufficient bioburden reduction to maintain Grade C/D environmental monitoring limits.

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The final sanitization process implemented leverages the data recovered from the baseline sampling along with the solution manufacturer coupon testing data. The routine regimen includes:

- Weekly – Quaternary ammonium RTU disinfection, followed by WFI wash of the windows
- Monthly – Quaternary ammonium RTU disinfection, followed by hydrogen peroxide/peracetic acid RTU sporicidal application, followed by a WFI wash of the windows
- Shutdown – Residue Removal Process – Hot WFI water wash, followed by 70% IPA wipe down

Data trending, including percent growth, percent excursion, growth maps, and heat maps, are used to evaluate the environmental results continuously. Changes in typical and seasonal flora, growth isolation/ location, and shifts in the bioburden gradient prompts a re-evaluation of the sanitization/disinfection program.

The study demonstrates the real-time efficacy of quaternary ammonium RTU and hydrogen peroxide/peracetic acid RTU used in Grade C/D clinical production environments. The study indicates growth levels are reduced consistently with the expected bioburden in terms of the magnitude within Grade C/D areas using the in situ efficacy test data. Following the site cleaning and disinfecting procedures for routine operations along with the triple cleaning steps related to production shutdown conditions, it is expected that the controlled conditions are established and maintained to viable particulate limits using quaternary ammonium RTU.

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Mr. Polarine is a senior technical service manager at STERIS Corporation. His current technical focus is microbial control in cleanrooms and other critical environments. He is a 2019 PDA Michael S. Korczynski Award recipient and he is a frequent industry speaker and published several PDA book chapters and articles related to cleaning and disinfection and contamination control. He is active on the PDA's COVID-19 Task Force and the PDA's Microbial Excursions Task Force. He was a co-author on PDA's Technical Report #70 on Cleaning and Disinfection. Mr. Polarine teaches at the PDA and the University of Tennessee. He is the current President for the PDA Missouri Valley Chapter and Technical Coordinator for the IEST. He is also a leader on the PDA's Chapter Council Steering Committee. Mr. Polarine graduated from the University of Illinois with a Master of Arts in Biology.
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