Vaporized Hydrogen Peroxide (VHP) Decontamination – Evaluation of Log Reduction for Biological Indicators and Other Considerations for Cycle Development

What Log Reduction is Acceptable for Cycle Development?

"Cycles should be developed with an appropriate margin of extra kill to provide confidence in robustness of the decontamination processes. ***Normally, a four-to six-log reduction can be justified depending on the application.*** *The specific BI spore titer used, and the selection of BI placement sites should be justified. For example, demonstration of a four-log reduction should be sufficient for controlled, very low bioburden materials introduced into a transfer isolator, including wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment."* [1]

Factors that May Influence Log Reduction Selection for Development of VHP Cycles

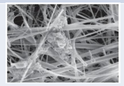
	Example #1	Example #2	Example #3
System Type	**Isolator system with glove ports** • No direct human intervention in critical zones except through glove ports • Risk of contamination – Breach of glove ports over critical zones (Medium)	**Closed Isolator system with no glove ports (robotic)** • No human intervention in critical zones during processing • No ability to breach through glove ports • Risk of contamination – Enclosed isolator (Low)	**Pass boxes** • Decontamination of smaller items for transfer into higher classification spaces. • Less criticality than a filling isolator as this is meant to transfer parts/materials which may undergo subsequent sanitization steps.
Surrounding Environment (Determination of the bio-load introduced into the area/system)	**Grade C vs. Grade D** • Gowning requirements are more stringent in Grade C versus Grade D. • Frequency of environmental monitoring may differ (number of data points) • Cleaning frequencies may differ There is more potential for a higher bio load in Grade D.	**Cleaning Program** • The robustness of the cleaning and disinfection program will influence the bio load present in the area. • Frequency of cleaning and disinfection and selection of agents.	**Microbial Baseline Flora** Understanding the types of microorganisms and bio load of your system as well as the surrounding environment is important when establishing VHP cycles. Performing a baseline study or reviewing historical EM data would be key in making this determination.
Cleaning of the System	**Establishment of Effective Cleaning Process for the System** • Robustness of the cleaning process for the system is important. VHP is a surface decontaminant, and residues are important to be removed between cycles/certain frequencies. • Considerations include finishes of surfaces to be cleaned, hard to clean/reach areas in the system, types of cleaning/disinfecting agents to be used based on the surface finishes. • Systems with harder to reach/clean areas may need to consider a more robust VHP cycle.		
Material Introduction into the System	**Assessment of Bio-load and Loading Pattern from Materials** • Material storage and transport prior to introduction (controlled area – less potential bioburden) • Sanitization process for materials prior to introduction into system • Number of materials – a larger load has more potential to introduce contamination and includes more surface area to decontaminate. • Are the materials in full contact with surfaces? More direct contact with surfaces that materials may have, the less likely the VHP will be able to reach these areas to decontaminate.		
Criticality of Activity being Performed in the System	**How Critical an Operation is for Patient and Product Safety** • Transfer isolator to move materials from one classification to another (lower criticality) • Isolator for aseptically filling products (higher criticality) • Sterilization of medical device (very high criticality)		

VHP Cycle Development with Traditional Biological Indicators

Biological Indicators (BIs), are test systems containing viable microorganisms providing a defined resistance to a specific sterilization process. A biological indicator provides information on whether necessary conditions were met to kill a specified number of microorganisms for a given sterilization/decontamination process, providing a level of confidence in the process.

Geobacillus stearothermophilus spores demonstrate a high resistance towards steam and vaporized hydrogen peroxide and are often chosen for cycle development purposes. **However, BIs can present some disadvantages**.

Disadvantages of Biological Indicator Use for VHP Cycle Development

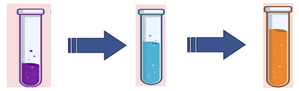
Factor	Disadvantage	
BI Results are Qualitative	Results are "Growth" and "No Growth", producing no quantifiable values for which to assess and adjust cycle parameters (dose/dwell time). There is no information provided on when the "kill" occurred.	
The Minimum Incubation Time to Achieve Results is 7 Days	This process is time consuming, especially if undesirable results are obtained and additional runs are required which adds to the overall cycle development timeline.	
Biological Indicator Manufacturing (Spore Distribution Concerns)	May cause 'false positive' results due to clusters and layers, which ultimately could cause adjustment of cycle parameters unnecessarily to achieve desirable results.	 [2]
Large Population Verification Variation Allowance	Population verification testing of the received batch of biological indicators allows for **50-300% variation** from the original population as stated by the manufacturer. There is a large amount of variability allowed.	
Overall Cost of Use	In large cycle development processes, including potential re-runs, up to 3 BIs are placed at each site location, which could add up cost quickly.	
Varying Storage Conditions	Storage conditions may vary from 2-8°C, to 20-25°C, dependent on the manufacturer. Humidity range (20-70%) storage which must be controlled. If stored incorrectly, could negatively influence results.	

Enzymatic Indicators (EI) – A Novel Approach to VHP Cycle Development [3]

Although Biological Indicators have been the standard for measuring success of cycle development processes, enzymatic indicators have the ability to provide similar data, but in a qualitative way.

Why tAK (**Thermostable Adenylate Kinase**)? This enzyme is very stable and displays a high tolerance to parameters such as high temperatures and oxidizing agents. Due to these reasons, tAK is the best EI to measure log reductions in a bio-decontamination process.

How do Enzymatic Indicators Produce a Log Reduction Result Similar to a BI?



Process Step	Step Explanation
Step 1 – Exposure	The EI(s) is exposed to the decontamination cycle. The EI is coated with an enzyme called tAK which becomes inactivated once exposed to the VHP.
Step 2 – Removal	The EI is removed from the system post-decontamination and inserted into the luminometer which produces a bioluminescent reaction that is measured in Relative Light Units (RLU).
Step 3 – Luciferase Injection	A reagent is injected (Luciferase) into the test tube containing the indicator. The purpose of this reagent is to act as a marker of the enzyme which will produce light.
Step 4 – ADP Injection	Another reagent called Adenosine Diphosphate (ADP) is injected into the test sample which measures how much of the enzyme is remaining after the decontamination cycle.
Step 5 – Conversion	tAK converts the Adenosine Diphosphate (ADP) into Adenosine Triphosphate (ATP).
Step 6 – Reaction and Measurement	The photometer captures the light reaction which presents itself in a "Relative Light Unit" measurement. The higher the value, the more enzyme is still present on the indicator strip which relates to how effective the decontamination cycle was. The lower the value, the less enzyme is present which correlates to a successful cycle. These values can correlate to a log reduction.

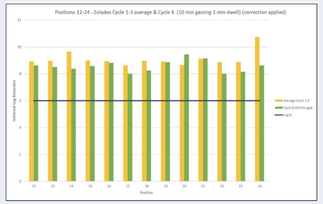
Advantages of Enzymatic Indicators during Cycle Development

Factor	Advantage	
Results are Quantitative	Values are produced from the processed EI in relative light units (RLU) which directly corresponds to the amount of enzyme remaining and can directly relate to log reduction. With a quantifiable result, cycles can be better adjusted to the optimal state.	
Rapid Results	Results are obtained in approximately 60 seconds!	
Low Variation	As opposed to biological indicators, the variation of EIs is <15%, providing more confidence in the results obtained.	
Less Time Consuming	With obtaining rapid results, there is less downtime in comparison to BIs which need to incubate for 7 days. As such, the cycle development process can be streamlined.	

AstraZeneca Study for Cycle Optimization Using EIs [4]

Original Cycle Parameters	Optimized Cycle Using EIs
Gassing (3g/min) – **15 minutes**	Gassing (3g/min) – **10 minutes**
Gassing Dwell (1g/ min) – **25 minutes**	Gassing Dwell (1g/ min) – **1 minute**
Aeration = **420 minutes**	Aeration = **180 minutes**

The original cycle development was performed utilizing biological indicators, for which long cycle times were developed based on the qualitative data. During optimization, EIs were used alongside BIs to provide a quantitative approach, which allowed to reduce overall cycle times and drastically reducing both "Gassing Dwell" and "Aeration" times.



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