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Parameters Effecting Vapor Hydrogen Peroxide BI Performance

by Eric Gillitzer, Ph.D.

Vapor Hydrogen Peroxide (VHP) under ambient pressure conditions is used in industrial settings to decontaminate aseptic fillings lines. In hospitals, VHP in a pre-vacuum modality is used mainly to sterilize instruments such as endoscopes and instruments that may be sensitive to high temperature steam. VHP can be considered, relatively speaking, to be a "new" method for sterilization/decontamination. The use of high temperature steam/moist heat, low temperature steam/moist heat, dry heat and ethylene oxide (EtO) as sterilization techniques have been around for decades. Hydrogen peroxide has only recently started to be used as a means of large-scale decontamination/sterilization.

Potential benefits of using VHP as a decontamination/sterilization media are: 1) VHP easily and quickly, given the proper conditions, breaks down into Oxygen (O_2) and Water (H_2O). Production of liquid hydrogen peroxide is easy and relatively inexpensive. 3) The compound apparently can be used at a variety of temperatures and humidity conditions. 4) No pressure vessel is required, so for large volume or area decontamination, such as rooms or entire buildings, hydrogen peroxide has proven very useful.

Some of the difficulties in using VHP as a decontamination/sterilization agent are: 1) For VHP, BI population does affect resistance. 2) VHP is not compatible with the cellulotic materials normally associated with BIs for steam, EtO and dry heat processes and alternative carriers must be used. 3) While the compound can be used at a variety of temperatures and humidity conditions, no set of standard exposure conditions exist to guide in the determination of resistance of BIs produced for this process.

Population

VHP as a decontamination/sterilization media has not been used as extensively as steam, dry heat or EtO and as such, much less is understood about parameters that affect BI performance. Population affects performance, but not in the same fashion as it affects BI performance in a steam process. With steam, dry heat and ethylene oxide, the measured resistance value is consistent regardless of the population given that the exposure conditions are otherwise kept the same. For example, the resistance of a strip BI used in 121°C steam cycle may be 1.8 minutes when assayed with an E6 population BI. Strips inoculated with the same crop but at a population of E5, E4 or E7 will test with a D-value of approximately the same 1.8 minutes. Experiments with dry heat, EtO and low temperature steam yield similar results. This observation, coupled with the fact that the spores behave with predictable behavior when the temperature is changed, we can not only use the same D-value with a lower population BI, but also calculate a *z* value (and thus estimate a D-value) for varying

conditions for these processes.

With spores on carriers used for VHP monitoring, the same IS NOT TRUE. Apex Laboratories, Apex NC, demonstrated with the production of the LOG-456 product, that the resistance of spores on a carrier does not stay constant as the population increases or decreases. The LOG-456 BI product consists of three carriers respectively inoculated with at least E4, E5 and E6 spores and sealed in separate compartments. Tables 1 & 2 show the resistance values for 9 lots of LOG-456 BIs produced in Bozeman, MT and Apex, NC. As we can see, as the population decreases, the resistance decreases. The explanation for this is quite simple. As killing of spores by VHP is not a penetrative process, the more closely placed or packed the spores are on the carrier, the less direct access the sterilant has to the spores. Thus, as the concentration of spores increases, the likelihood of a spore being in direct contact with another spore increases.

Table 1. LOG-456 Lots

Lot	Produced	Population	Resistance (min)	Lot	Produced	Population	Resistance (min)
P1271	Bozeman, MT	E4	0.3	P3170	Apex, NC	E4	0.3
		E5	0.6			E5	0.6
		E6	0.9			E6	0.9
P1231	Bozeman, MT	E4	0.3	P3230	Apex, NC	E4	0.7
		E5	0.6			E5	0.8
		E6	0.9			E6	1.0
P1841	Bozeman, MT	E4	0.7	P0340	Apex, NC	E4	0.2
		E5	0.8			E5	0.2
		E6	1.0			E6	1.0
E5 E6				P1540 0.9 1.3	Apex, NC	E4	0.3
P0930	Apex, NC	E4	0.3	P0651	Apex, NC	E4	0.4
		E5	0.6			E5	0.8
		E6	1.4			E6	1.0

Table 2. LOG-456 Lots population and resistance

Summation of Resistance Data	
Population	Resistance (min)
E4	0.2, 0.3, 0.3, 0.3, 0.3, 0.3, 0.4, 0.7, 0.7
E5	0.2, 0.6, 0.6, 0.6, 0.6, 0.8, 0.8, 0.8, 0.9,
E6	0.9, 0.9, 0.9, 1.0, 1.0, 1.0, 1.0, 1.3, 1.4,

With regard to spores touching each other or stacking on the surface of the inoculated material, one must understand that this is always going to happen. This occurs in a somewhat predictable or repeatable fashion with a smooth, 2-dimensional carrier such as a stainless steel disc. Because of the nature of the stainless steel surface, there is ALWAYS going to be a ring effect at the edge of the inoculated area. The formation of the ring affect may be more of an issue with a flat surface (Apex spore ribbon) than with an angled surface (Apex spore disc), but it is always going to be present.

With a 3-dimensional carrier such as fiberglass, it is easy to imagine spores penetrating into the matrix of the carrier and then aggregating as they are filtered out of suspension by the fibers of the carrier. This can happen

with both the paper and fiberglass carriers. With steam, EtO and dry heat, the sterilant is able to easily penetrate into both the matrix and any spore aggregates that may have formed during the inoculation process. VHP may penetrate the matrix of the carrier, but as discussed earlier, is not able to penetrate into aggregates of spores that may have formed as part of the inoculation process. There are at least two ways to easily deal with this. One would be to use a cycle that is a bit more aggressive or longer in order to try to get some penetration through layers of spores that may exist in these sorts of areas. A second method to deal with this would be to use a BI with a population of E4 or E5 rather than E6. Less spores means less potential for stacking or aggregation and potentially easier killing.

With regard to the use of an E5 or E4 BI rather than an E6 BI, the use of an E6 BI may be an arbitrary choice with respect to surface decontamination. The use of an E4, E5, or a modified E6 BI should allow the end user to run cycles that would be lethal enough to kill the BI with relative ease, and may not display the number of "rogue" BIs currently reported simply because the packing density of spores would be reduced. Realistically, as many of the isolators decontaminated with VHP are run almost daily and everything going into the isolator has been sterilized or decontaminated, the potential for the introduction of a large amount of contamination is quite small. Furthermore, if a contaminant were present, it would more than likely be present at a very low level and be present as a non-spore forming contaminant. Thus, the use of a lower population BI for surface decontamination in these sorts of environments may be warranted.

Peroxide concentration and other factors

Another source of frustration with the VHP process is deciding what concentration of peroxide to use. Over time with steam, dry heat and EtO processes, a standard set of conditions came into being. For steam sterilization, the standard condition is 121°C saturated steam. For EtO, 60% humidity, 600 mg/L EtO at 54°C is the standard condition. There is no standard condition for VHP. The Apex product Certificate of Assurance states the resistance testing is done at 2 mg/L of peroxide. Many end users use less peroxide than this in their testing and use cycles. Other possible impacts on process performance could be variances in the vapor or airflow rate between isolators, variances in the turbulence between isolators and finally variances in temperature and humidity between isolators. Again, there are no standard set of conditions for BI performance that have been dictated by the guidance documents, and ISO 18472:2006 Section 4.8 details only the instrumentation requirements for a peroxide resistometer.

Similar performance affects with chemical sterilants

The performance of the most commonly used chemical sterilant, EtO, can be impacted by both the relative humidity during exposure and the temperature. We have observed that given the same temperature and sterilant conditions, when low RH conditions are used during an exposure BIs that would have been killed are shown to survive. Temperature during exposure does have an effect on the resistance, as ISO 11138-2:2006 states that EtO D-values may have two different ranges if the exposure temperature is different. In testing Chlorine Dioxide BIs here at our facility, we have determined that both temperature during exposure and relative humidity during exposure affect BI performance. With these observations in mind, that temperature and humidity can and does affect the efficacy of killing with other chemical sterilants, it seems plausible that the killing efficacy with VHP could likewise be influenced by variations in these same parameters.

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Eric is a member of the Association for the Advancement of Medical Instrumentation (AAMI) and a former member the American Society for Virology (ASV) and American Society for Microbiology (ASM). Eric graduated from Montana State University, Bozeman with a B.S. in Microbiology and from SUNY Stony Brook with a Ph.D. in Molecular Biology and Biochemistry.