

BIT™ Detailed Technical Discussion

Hydrogen peroxide is a strong oxidizer that is used for high-level disinfection and sterilization. It produces reactive hydroxyl free radicals and ions that can attack membrane lipids, DNA, and other essential cell components of microorganisms. A 3% to 6% solution is conventionally used for an exposure time of 10 to 60 minutes depending on the microorganism targeted. In general, vegetative bacteria are most susceptible and bacterial spores the most resistant. Concentrations of 3% to 6% have been used for the disinfection of ventilators, soft contact lenses, and tonometer biprisms. However, liquid disinfection with hydrogen peroxide is not widely used in the medical industry because of lengthy sterilization times. Recently, vaporized hydrogen peroxide has been used for low-temperature sterilization by way of a plasma sterilizer. Two plasma sterilizers are commercially available, the Sterad 100 sterilizer (Advanced Sterilization Products – a Johnson & Johnson Company) and the Plazlyte sterilizer, both require a vacuum and high concentrations of hydrogen peroxide to operate. The low pressure plasma in the vacuum environment induces free radicals and ions, which enhance the bactericidal process. These systems are utilized for sterilizing surgical instruments, and they require extensive containment enclosures.

Plasma: The three common forms of matter that we experience on earth are solids, liquids and gases. However, the fourth state of matter, plasma, comprises 99% of the visible universe.

Sir William Crookes, an English physicist, first identified plasma in 1879. However, the word “PLASMA” was not applied to ionized gas until 1929, by an American chemist, Dr. Irving Langmuir. Plasma consists of free-moving electrons and ions that can be formed when energy is applied to molecules, e.g.: $H_2 + \text{energy} \rightarrow 2 H^+ + 2 e^-$. Many different forms of energy can be used: thermal, electrical, light, etc. With insufficient sustaining power, plasmas recombine into neutral gases, liquids or solids. Examples of common plasmas include the sun and stars, lightning, a fluorescent or neon light, gas lasers, vacuum tube discharge, etc.

BIT™: Binary Ionization Technology™ (BIT™), developed by Intecon (US Patent # 6,343,425), is the process of passing a cleaning and disinfecting mist through plasma (ionized gas) which results in a more effective and drastically shorter disinfection / cleaning process. The chief advantage of BIT™ over previous H_2O_2 / plasma systems is that BIT™ works at standard atmospheric conditions and does not require a vacuum (i.e. BIT™ can be used in the open air without special enclosures for containment).

A weak solution of hydrogen peroxide was chosen as the first system to evaluate since it is a chemical that is commonly used in cleaning and disinfecting. Hydrogen peroxide is relatively inexpensive, leaves no residue, and is effective in disinfecting open wounds. The reactivity of hydrogen peroxide is easily seen in the foaming that occurs when it is applied to an open wound. The foaming occurs because the hydrogen peroxide disassociates into water and oxygen in the presence of enzymes present in open wounds. However, hydrogen peroxide is known to be relatively slow in disinfecting. At ambient temperatures and pressure, 20 minutes of contact is recommended to disinfect a wound.

The BITTM reaction functions in a way similar to vacuum based H₂O₂/ plasma systems – the plasma efficiently transfers energy and activates the hydrogen peroxide. The activated species then kill microbes and recombine to water and oxygen. The difference between BITTM and vacuum based H₂O₂/ plasma systems is that BITTM uses a fluidized bed of liquid particles instead of a vacuum generated vapor, thus creating a significantly larger reservoir of H₂O₂ molecules to react. BITTM also utilizes “atmospheric cold plasma” technology that allows the creation of suitable plasma without a vacuum.

The plasma-activated species of hydrogen peroxide (ions, hydroperoxy, hydroxyl or other free radicals, excited hydrogen peroxide molecules, etc.) are extremely effective at killing microbes. The initial studies, which demonstrate this, were performed with *Serratia marcesens*, a Gram negative bacillus. This organism was selected for two reasons. It is the organism specified in the ASTM “Standard Method for Evaluation of Health Care Personnel Handwash Formulation”. It is also a difficult organism to kill in a system using hydrogen peroxide as it contains high cellular catalase activity. The first experiment, presented on the next page, showed that hydrogen peroxide without the plasma has no detectable effect in 60 seconds while the same application through the plasma field gave 6 log reduction in 60 seconds (see Table 1). The next experiment shows that the level of kill exceeds a 6 log reduction, the level typically used to indicate sterilization potential, with just 15 seconds of spray (see Table 2). The final experiment with *S. marcesens* again shows that extremely high efficacy of activated hydrogen peroxide at very low concentration, 0.3%, in 15 seconds (see Table 3).

More recent studies have been performed using *Bacillus subtilis*, an accepted Anthrax surrogate, and *Bacillus stearothermophilus*, which is very resistant to hydrogen peroxide. As shown in the pictures and table, Binary Ionization TechnologyTM using 3% hydrogen peroxide through a 10.5 Kv plasma arc for 15 seconds was very effective at killing both spores while hydrogen peroxide alone was not effective (see Table 4).

The Effect of Plasma on Hydrogen Peroxide Efficacy

Test Protocol: *S. marcescens* was plated on to a 0.45 μ membrane filter in varying concentrations. A solution of USP hydrogen peroxide was sprayed through a plasma and directed toward the filter, which was held at a constant distance of 12.5 inches. D.I. Water was used as a control and solution is sprayed with and without the plasma.

Plasma:	No	No	Yes
Solution:	<i>D. I. Water</i>	3% H₂O₂	3% H₂O₂
Exposure Time:	60 Seconds	60 Seconds	60 Seconds
Initial CFU Conc.	Final CFU Conc.	Final CFU Conc.	Final CFU Conc.
4.3×10^2	Too Numerous to Count	Too Numerous to Count	0
4.3×10^3	Too Numerous to Count	Too Numerous to Count	0
4.3×10^4	Too Numerous to Count	Too Numerous to Count	0
4.3×10^5	Too Numerous to Count	Too Numerous to Count	0
4.3×10^6	Too Numerous to Count	Too Numerous to Count	3

(Table 1)

Conclusion: *The plasma arc “activates” the hydrogen peroxide, enhancing the efficacy.*

The Effect of Time on BIT™ Efficacy

Test Protocol: *S. marcescens* was plated on to a 0.45µ membrane filter in varying concentrations. A solution of USP hydrogen peroxide was sprayed through a plasma and directed toward the filter, which was held at a constant distance of 12.5 inches. The exposure time for the solution was compared at 60 and seconds.

Plasma:	Yes	Yes
Solution:	3% H ₂ O ₂	3% H ₂ O ₂
Exposure Time:	60 Seconds	15 Seconds
<u>Initial CFU Conc.</u>	<u>Final CFU Conc.</u>	<u>Final CFU Conc.</u>
2.5 x 10 ²	0	-
2.5 x 10 ³	0	0
2.5 x 10 ⁴	0	-
2.5 x 10 ⁵	0	0
2.5 x 10 ⁶	0	0

(Table 2)

Conclusion: *A 6-log reduction was achieved in 15 seconds using 3% hydrogen peroxide and a 10.5 KV plasma arc.*

The Effect of Hydrogen Peroxide Percentage on BIT™ Efficacy

Test Protocol: *S. marcescens* was plated on to a 0.45µ membrane filter in varying concentrations. A solution of USP hydrogen peroxide was sprayed through a plasma and directed toward the filter, which was held at a constant distance of 12.5 inches. The exposure time for the solution was 15-seconds with the concentrations of H₂O₂ dissociated through a 10.5 KVAC plasma arc varied between 3.0% and 0.3%.

H ₂ O ₂ Conc.:	3%	1.5%	0.75%	0.3%
Exposure Time:	15 Seconds	15 Seconds	15 Seconds	15 Seconds
<u>Initial CFU</u> <u>Conc.</u>	<u>Final CFU</u> <u>Conc.</u>	<u>Final CFU</u> <u>Conc.</u>	<u>Final CFU</u> <u>Conc.</u>	<u>Final CFU</u> <u>Conc.</u>
2.3 x 10 ²	0	0	Minor (edge only)	Minor (edge only)
2.3 x 10 ³	0	0	0	Minor (edge only)
2.3 x 10 ⁴	0	0	0	Minor (edge only)

(Table 3)

Conclusion: Extremely low levels of hydrogen peroxide through plasma are very efficacious.

The Effect of BIT™ on Resistant Organisms

Test Protocol: A solution of USP hydrogen peroxide was sprayed on a spore strip containing an anthrax surrogate and a hydrogen peroxide resistant spore. The solution was held at a constant distance of 12.5 inches and sprayed for 15 seconds. D.I. Water was used as a control and solution is sprayed with and without the plasma.

Hydrogen Peroxide:	None	3%	3%
Exposure Time:	15 Seconds	15 Seconds	15 Seconds
10.5 Kv Plasma:	No	No	Yes
<u>Initial CFU</u>	<u>Final CFU</u>	<u>Final CFU</u>	<u>Final CFU</u>
4.3 x 10 ⁶ B. subtilis	Too Numerous to Count	Too Numerous to Count	None
4.3 x 10 ⁶ B. stearothermophilus	Too Numerous to Count	Too Numerous to Count	None

(Table 4)

Conclusion: BIT™ is effective against an Anthrax surrogate and hydrogen peroxide resistant spores.