

Validation Study:

PERASAN[®] Efficacy Against *Listeria monocytogenes* and *E. coli* in 15% Salt Brine Solutions at 40° F

Background: Meat, poultry, seafood and dairy industries often utilize brine chilling operations to cool or prepare the end product for final packaging. Brine (salt) solutions are preferred in many cases to preserve the osmotic balance between the finished product and the cooling water medium. The products subject to brine chilling may include ready-to-eat or further processed products of all kinds, shapes and ingredients. In an effort to prevent the spread of harmful organisms such as E.coli, Pseudomonas, Listeria, Salmonella, etc, most processing companies employ some kind of antimicrobial or inhibitory treatment. This antimicrobial treatment must exhibit a reasonably good half-life in concentrated brine solutions, be non-corrosive to the materials of construction, be efficacious against target organisms in very cold solutions, and must be FDA approved for direct food contact without requiring a potable water rinse. The only chemical that meets all these requirements is peracetic acid, which is also known as peroxyacetic acid. Thus, our goal in this evaluation is to validate the efficacy of Enviro Tech's PERASAN[®] Antimicrobial solution using several challenge (target) organisms under in-use conditions.

PERASAN[®] is an equilibrium concentrate of 15% peracetic acid (PAA), 6% hydrogen peroxide, and minor amounts of acetic acid and a stabilizer. The product is a very powerful oxidizer. PAA is a more powerful oxidizer than chlorine dioxide and second only to ozone at near neutral pH's (6-8). In addition, it is quite reactive to microbiological fauna, yet is selectively tolerant to many types of organic matter, fats, and oils typically associated with food processing operations such as meat, poultry, and seafood. Since PERASAN[®] contributes no conductivity to the wastewater stream and does not persist in wastewater treatment operations, it is an ideal candidate for use as an antimicrobial treatment for chilled water or brine-based solutions.

Evaluation and Considerations: Peracetic acid (PAA) is quite unique in its broad spectrum use. It is well known that PAA solutions are degraded very rapidly by seawater, typically expressing a half-life of less than 3-5 minutes. However, in another paper disclosed previously by Enviro Tech, it was discovered that PERASAN[®] PAA had surprising stability in 15% salt brine solutions at room temperature. Many antimicrobial treatments perform poorly in cold water, and it was determined to be prudent to challenge PERASAN[®] in these reduced temperature regimes against the pathogenic organisms E. coli and Listeria monocytogenes using low concentrations (which are very economical to justify for the end-user). These low concentrations would also represent a "worst case" scenario regarding the resulting efficacy of these peroxyacetic acid solutions against these pathogenic organisms.

Methods: 150 grams of non-iodized salt was added to 850 gms of tap water to yield a 15% w/w salt brine solution. ATCC strains of *E. coli* and *Listeria monocytogenes* were incubated on appropriate nutrient agars to yield colony counts that were at least 1×10^8 in numeration. The brine solution was pre-chilled to 38-41° F, to which were added serial dilutions of PERASAN® to yield PAA concentrations of approximately 15 ppm (as peroxyacetic acid activity). Subsequently, aliquats of the respective challenge organisms were added to the brine-PAA solution, and samples of the resulting mixture were extracted and neutralized at intervals of 1, 5, 10, 15, 20, 25, and 30 minutes. The neutralization process consisted of adding 225 ppm of pH adjusted sodium metabisulfite, which is sufficient to consume all the combined peracetic acid and hydrogen peroxide.

All subsequent microbiological plating was done using the organism-specific appropriate 3-M Petrifilm plates and incubated at 35°C. Duplicate plating was the standard in all cases, and neutralization controls were performed using non-PAA treated/neutralized challenge organisms as the confirmation of no interference of the neutralizer or brine.

Results: When challenged against *E. coli*, 15 ppm active PERASAN® resulted in surprisingly effective and rapid destruction of this organism. The microbiological results yielded zero growth in 1 minute from a challenge concentration of 1×10^5 cfu/ml.

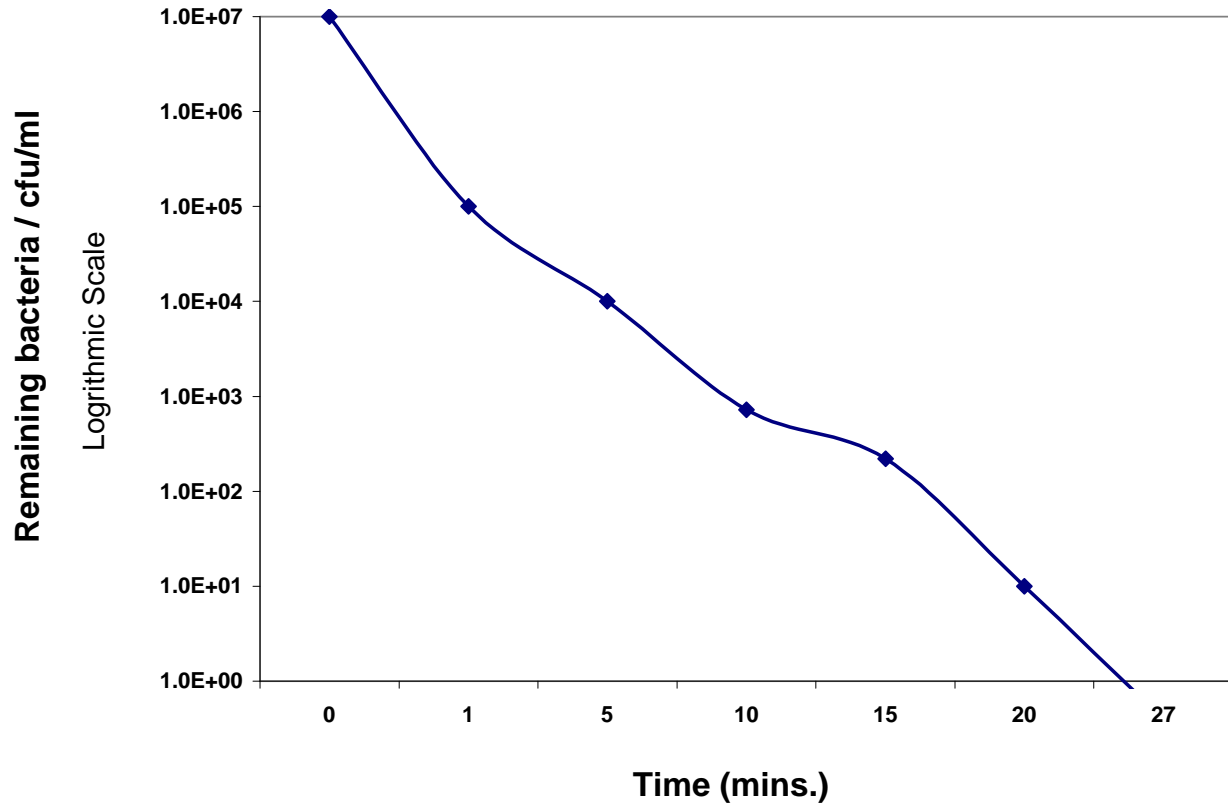
Listeria monocytogenes appears to be a much hardier organism. Zero growth was not apparent from the 1×10^7 challenge until 26 minutes into the exposure. Growth was detected at the 25 minute interval, and this specific challenge was re-run (using identical conditions) to establish the exact time line between the 25 and 30 minute intervals. This duplicate challenge served as a confirmation of the resistance of the *Listeria* species to 15 ppm PAA, and established the time line for complete kill at 26 minutes.

During all the challenge exposures the activity of the peroxyacetic acid concentration was recorded. The PAA residual was determined throughout the test challenge at each time interval, and the concentration remained above 11 ppm in all cases.

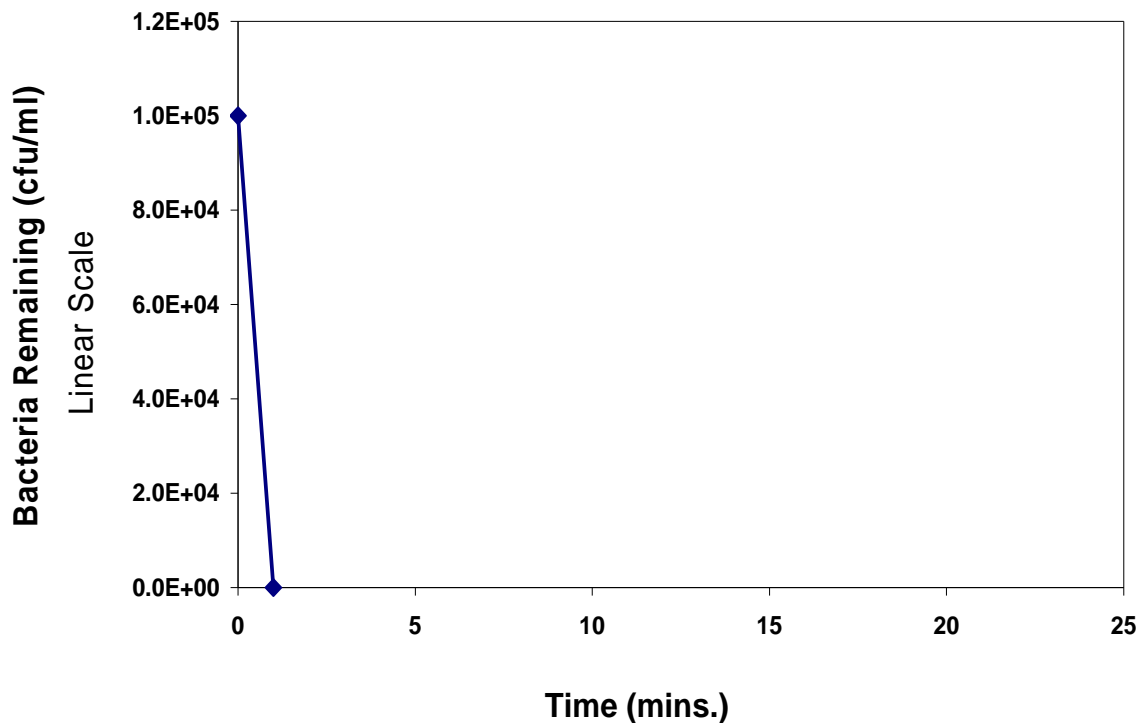
The results are displayed on the following two graphs.

Conclusion: PERASAN® appears to be a very viable option for chilling and brine-cooling food contact situations. The product is approved by the FDA in 21 CFR 173.315 and 21 CFR 173.370 (among other citations) for use in *direct* contact situations for fruits, vegetables, meat, poultry and seafood applications that do not require a water rinse. PERASAN® has outstanding efficacy against *E. coli* at low concentrations, and has 100% kill abilities against high concentrations of *Listeria monocytogenes* within 26 minutes under the conditions tested.

Efficacy of PAA (15 ppm) against *Listeria monocytogenes* in 15% salt brine @ 40°F



**Efficacy of PAA (15 ppm) against E. coli
in 15% salt brine @ 40°F**



Note: The results reported herein are part of intellectual property (patented) uses that have been or are being developed by Enviro Tech, Inc. Modesto, CA. This public disclosure does not waive any rights or issue conveyance of intellectual property from Enviro Tech to any person or user of Enviro Tech's peroxyacetic acid-based applications described herein without expressed written consent of Enviro Tech, Inc.

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