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Laboratory Certification
AIHA LAP #183867
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Experimental Briefing: Rapid Degradation of *Legionella pneumophila* and the H1N1 Flu Virus in CAZ Solution.



Product Testing Lab
May 16, 2017

Overview

- Assured Bio Labs, LLC was contracted by Clean Air Zone (CAZ) to conduct time series analysis to determine the capacity of CAZ to degrade viable Legionella pneumophila cell and infective H1N1 flu virus particles.
- A mixture of H1N1 and Legionella pneumophila was prepared at a 7 Log concentration. The mixture was inoculated at a 1:100 dilution into three different solutions.
 - 80% CAZ Solution
 - 80% CAZ Solution Autoclaved (100°C @ 19psi for 30 minutes) to kill all microbes present in the CAZ solution.
 - Tap Water
- The inoculated CAZ and water solutions were aerated during incubation by gently mixing on a Glass Col. multiwell plate mixer at a 65 rpm.
- Samples were collected for DNA analysis immediately following inoculation (T₀) and every 12 hours thereafter (T₁...T₉).

Key Findings

- DNA analysis allowed for the detection and quantification of the specific target microbes (Legionella and H1N1) when added to the complex microbial CAZ Solution mixture. DNA analysis uses high fidelity polymerase chain reaction technology that has superior detection range compared to standard microbial culture analysis.
- Legionella pneumophila DNA was significantly reduced to a 2 log concentration within 72 hours (see Figure 1).
- H1N1 viral DNA was immediately degraded when added to the 80% CAZ Solution. Estimated time from addition to T₀ sample processing was 40 minutes (see Figure 2).
- Extensive residual activity persisted in the CAZ solution after autoclave sterilization. The findings indicate that even when the living fraction of the CAZ solution is killed, the enzymatic fraction continues to function and is capable of neutralizing pathogenic microbes.

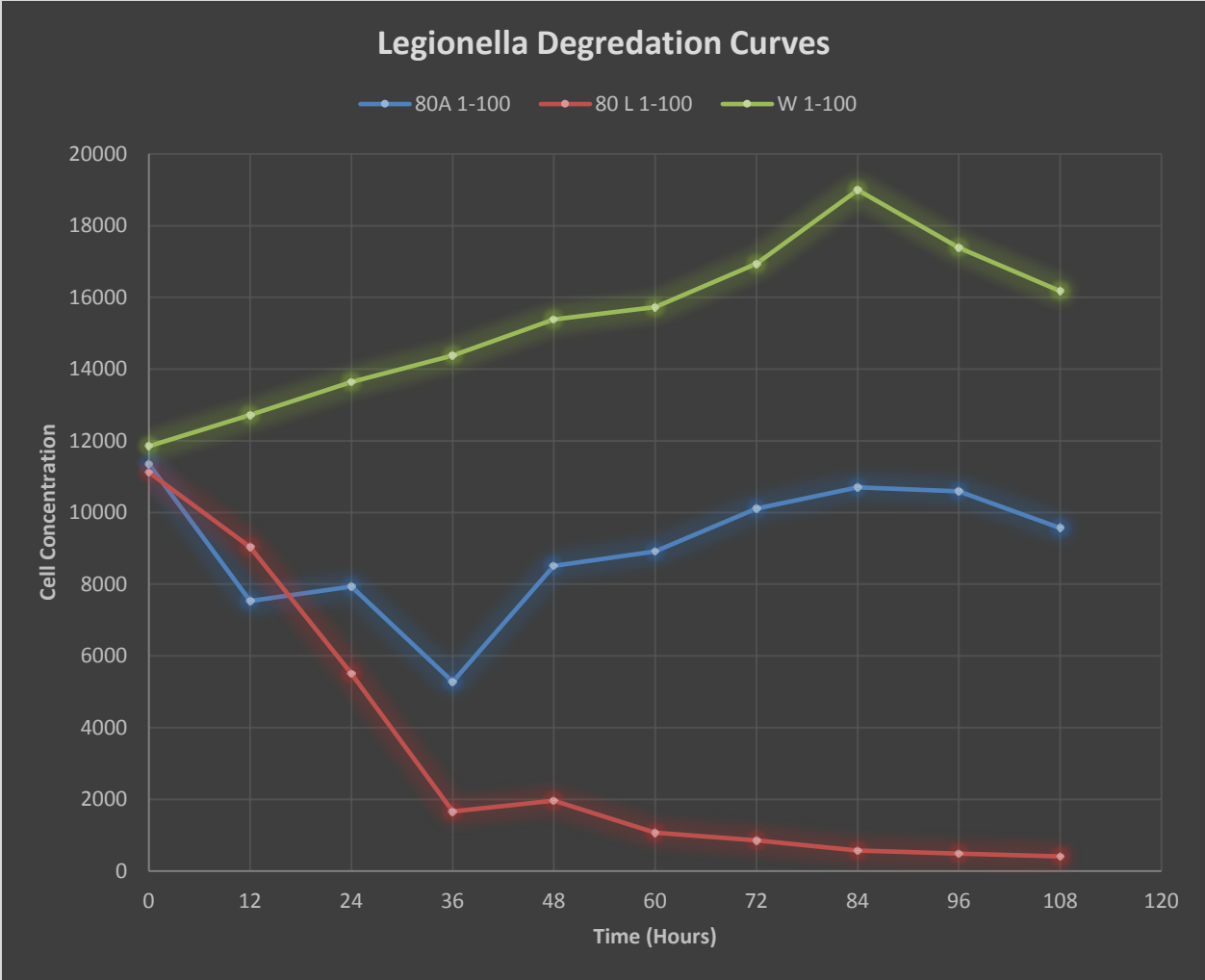


Figure 1. Rapid degradation of Legionella is observed in 80% CAZ Solution (red line) within 72 hours following inoculation. Slight variation occurred in the Legionella concentration in tap water (green line) which was either due to slight Legionella growth in tap water or cell clumping during sampling. Little change was observed in Legionella concentration in the sterilized 80% CAZ solution (blue line).

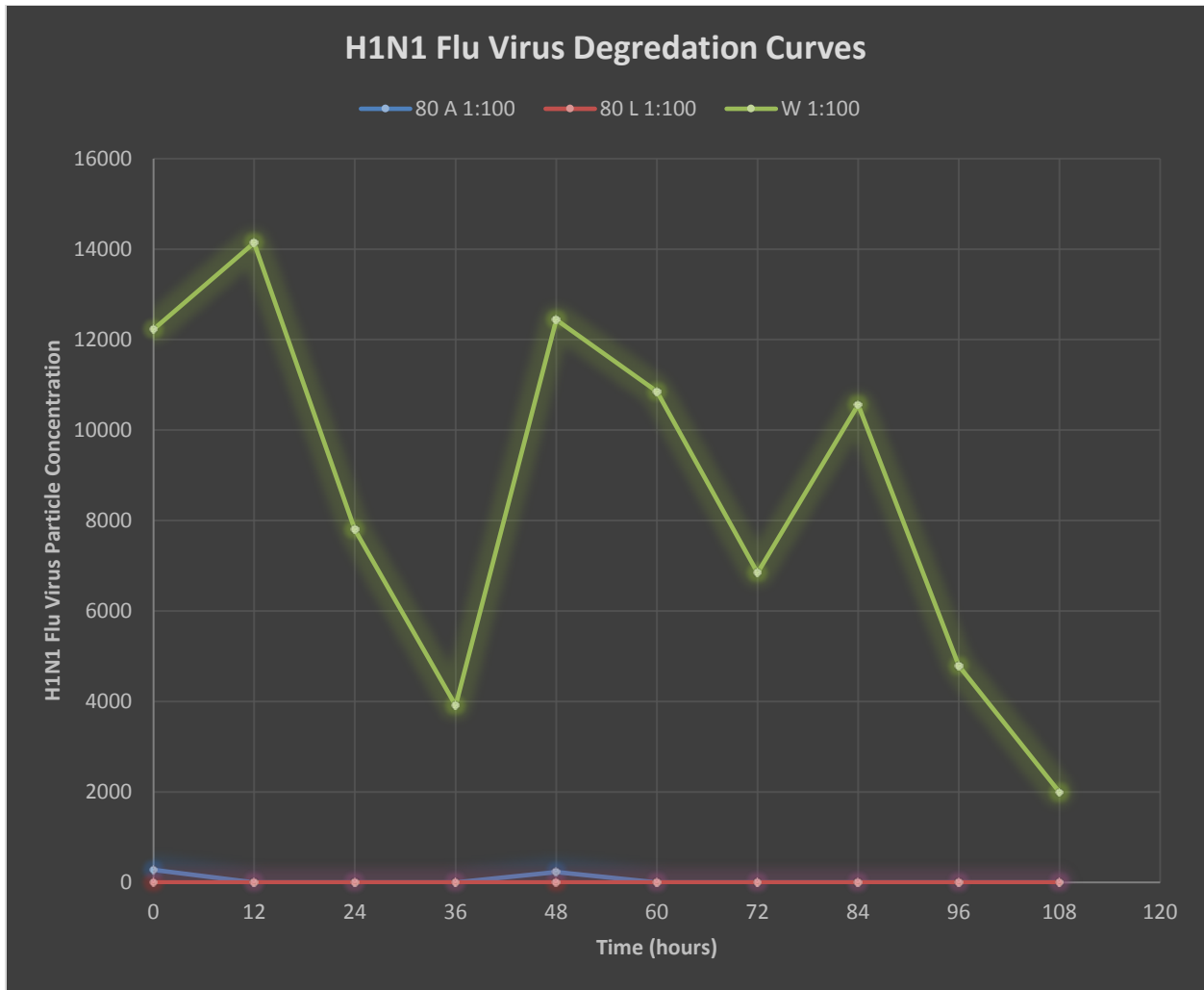


Figure 2. Rapid degradation of H1N1 Virus is observed immediately upon inoculation into 80% CAZ Solution (red line). The virus is estimated to have resided in the CAZ solution for approximately 40 minutes before sample processing was initiated. The H1N1 concentration in tap water (green line) fluctuated until a large decline occurred at 96 hours. The H1N1 concentration also was largely degraded immediately after inoculation into the sterilized 80% CAZ solution (blue line); however some virus persisted up to 48 hours.