

Survival of VBNC *Legionella pneumophila* in drinking water following chlorine disinfection

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Legionella pneumophila is a waterborne pathogen that has been found to be resistant to the chlorine disinfection of drinking water. The aim of this work is to demonstrate that although no *L. pneumophila* can be detected by using standard plating techniques, VBNC cells can still survive in water systems.

A cell suspension of *L. pneumophila* NCTC 12821 was prepared in dechlorinated and filter-sterilised drinking water. Chlorine was added and samples were taken after 0, 10, 20 and 30 minutes for cell quantification by culture on BCYE agar plates and by SYTO 9/PI double staining.

In the control assay (with no chlorine) the number of cultivable and viable cells remained constant with time. When exposed to 0.2 mg L⁻¹ of chlorine it was observed that cell cultivability decreased with time, but the number of viable cells remained constant. When chlorine concentration was increased to 0.7 mg L⁻¹ and 1.2 mg L⁻¹, cultivability was completely lost in 30 and 10 minutes, respectively. In both cases there was no significant decrease in the number of viable cells and no DNA cuts were detected by DNA electrophoresis.

These results demonstrate that even after completely losing cultivability, it is possible to find live cells in drinking water in the VBNC state. This suggests it is necessary to employ new methods to detect pathogens in water after disinfection to guarantee a safe distribution of drinking water.