# Improved methods show survival of *E. coli* O157:H7 in potable water biofilms following treatment with high chlorine concentrations

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Biofilm structures can provide protection against both mechanical and chemical attack and thus, can act as a reservoir for pathogens. *Escherichia coli* O157:H7 has been associated with a number of serious waterborne outbreaks. It has been shown to be able to persist and survive within biofilms on pipe and tank walls for long periods of time. It is essential that disinfectant and decontamination procedures are able to effectively target pathogens which are incorporated into biofilms as well as those in the planktonic phase. It is well documented that standard culture methods can underestimate the numbers of viable cells due to an inability to detect bacteria in a viable but non-culturable state (VNC).
In this work, a combination of a specific peptide nucleic acid (PNA) probe has been used in a fluorescence *in situ* hybridisation (FISH) assay with cell elongation. PNA probes have many advantages over standard DNA probes and have been shown to be effective at detecting low levels of pathogens in complex samples. Using the episcopic differential interference contrast/epifluorescence (EDIC/EF) microscope, we are able to examine the biofilm *in situ* and visualise individual target cells. PNA-FISH does not give a reliable measure of viability and so we combine this approach with a cell elongation assay which uses the antibiotic, pipemidic acid. Pipemidic acid inhibits cell division resulting in highly elongated cells if viability is maintained. Using a multi-stage chemostat system to model a drinking water supply, we have generated biofilms on PVC coupons and spiked the system with *E. coli* O157:H7. The action of the disinfectant chlorine has been studied at concentrations ranging from low residual amounts to 100 ppm.
Using this approach, it was possible to detect elongated *E. coli* O157:H7 following all disinfectant treatments up to 100 ppm, even though no counts were obtained from standard culture methods. This has serious implications for efficient and effective water treatment protocols.