Comparison of Methods for the Detection of Culturable and VBNC *E. coli* O157:H7 in Complex Drinking Water Biofilms

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E. coli O157:H7 is a serious pathogen, causing outbreaks worldwide and has been involved in waterborne transmission such as Walkerton, 2000. Standard detection methods rely on culture techniques but these have several limitations; having been shown to be ineffective at detecting viable but non-culturable (VBNC) bacteria or those located in biofilms. In this work, culture methods have been compared to the use of cell elongation combined with peptide nucleic acid (PNA) probes in a fluorescence in situ hybridization (FISH) assay and a real-time PCR (qPCR) technique utilizing propidium monoazide (PMA). These methods assess viability as well as permitting sensitive detection.

A PNA probe which specifically labels the 16S rRNA of *E. coli* was synthesised with a fluorophore label and used in a FISH assay. Cell elongation, which involves the use of the antibiotic pipemidic acid (which inhibits cell division), was applied prior to PNA-FISH and the presence of elongated, labeled bacteria indicated the presence of viable *E. coli* which were then quantified by epifluorescence microscopy (EF). In addition, the use of episcopic differential interference contrast microscopy (EDIC) allowed the visualization of labeled cells directly within a biofilm structure. The molecular technique of PMA-PCR, uses qPCR to quantify the target population and PMA allows separation of the non-viable

proportion of the population. PMA-PCR is dependent on comparison with a direct approach to convert the obtained Ct values to an actual population measure.

In Figure 1, comparisons (for total cell count and *E. coli* O157:H7 only) between culture analyses and microscopy labeling methods are shown. It is clear that the use of SYTO 9 for total cell counts and cell elongation with PNA-FISH, detect a higher population density than culture analyses alone. This suggests that VBNC bacteria are also being detected and quantified. As advanced molecular methods, such as PMA-PCR, rely on actual counts to produce a calibration curve, it is essential that an accurate technique be used.

This study indicates the requirement for improved detection methods, which will detect VBNC bacteria and can be used with complex sample types. Biofilms can act as a reservoir for serious pathogens, including *E. coli* O157:H7, and outbreaks will continue until such methods are improved.

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Keywords: E. coli O157:H7, biofilms, drinking water, PNA-FISH, PMA-PCR

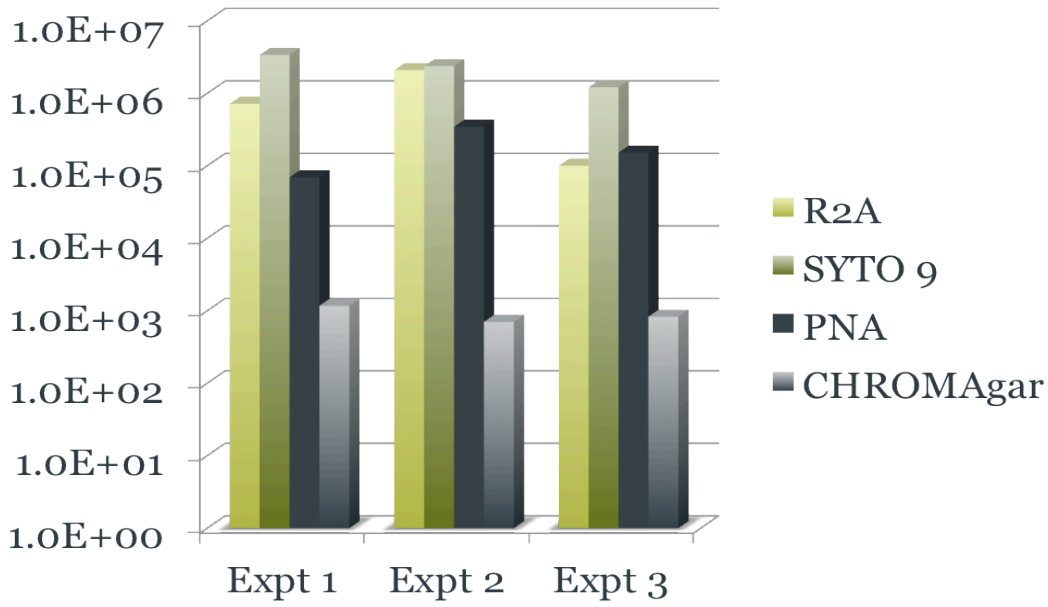

Figure 1. Comparison of R2A, SYTO 9, PNA (with cell elongation) and CHROMagar O157 counts for biofilm spiked with *E. coli* O57.