Rapid confirmation of microbiological alerts using off-line molecular methods

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Summary of presentation/poster

In the event of a microbiological alert, either due to the triggering of an on-line sensor or malicious threats made to the news media, there is an urgent need to rapidly confirm the identity and quantity of the pathogen or indicator species involved in the emergency. If positive, then immediate decisions can be taken with confidence to quarantine the water and/or issue a boil notice to protect the public health. Existing isolation and culturing methods are time consuming, taking as long as 2-3 months for slow growing pathogenic species, or may not work for those environmentally stressed species in a viable but non-cultivable (VNC) state. Consequently, rapid identification will most likely entail application of off-line detection methodology, utilising state of the art molecular techniques, for contaminants in water and on distribution network pipe surfaces following the probable adsorption of some of the agent. An important requirement is that such methods are not only sensitive and specific, avoiding false negative or false positive reports, but also they are robust: it is not sufficient that such methods work under laboratory conditions, they must be capable of working in diverse water chemistries such as high humic acid or iron concentrations, and with complex pipe deposits. If necessary, the agent must be successfully separated from the pipe deposits before analysis can be completed, requiring improved methodologies for sample preparation. This presentation will review progress of rapid detection and viability assessment methods, including a critical assessment of the importance of the viable but non-cultivable environmentally stressed phenotype in normally treated distribution systems and following remediation strategies. This knowledge will provide reassurance to the authorities that pathogens have indeed been removed and/or killed following decontamination interventions, and that the water distribution system can be signed off as being fit to return to normal use. The methods to be appraised will include:

i) Fluorescence *in situ* hybridisation (FISH) using DNA probes or the more recent robust PNA probes, designed to hybridize with 16S or 23S rRNA, even in heavily corroded pipe deposits. This technique can be used with filter-capture of the biological agent from the bulk water, which provides a useful concentration step, or with recovered pipe deposits. A further refinement is that it can be subsequently coupled with a method to specifically detect viable cells following disinfectant treatment by following growth of captured cells over a short incubation period.

ii) PCR and qPCR, coupled with the recent advance of incorporating propidium monoazide (PMA) into the sample assay to determine the viability of a cell or spore without culture. PMA intercalates with DNA, preventing PCR amplification of target sequences but it cannot pass through the membranes of healthy, living cells; only dead cells. Therefore dead cells are not detected by PCR or qPCR and the qPCR data obtained in the presence and absence of PMA provides information on the percentage of live or dead cells of the specific agent present.

Examples of the microbiological agents used to validate the methods will be provided from the ongoing EC FP7 *SecurEau* project.