METHOD STATEMENT

Determinand:
BD Accuri C6 Flow Cytometry

Matrix:
Sample Type: drinking, ground and surface waters

Principle of Method:
Flow cytometry offers a powerful and effective method for assessing bacteria in water samples. Particles or cells suspended in a hydrodynamically focused liquid stream pass through a pulsed beam of laser light. Optical detectors collect scattered laser light and fluorescent emissions, and electronics digitize the signal for computational analysis. The light scatter data provides basic information about the cells such as relative size, shape and surface features. The fluorescence data reveals the autofluorescence and/or labelling with fluorescent dyes, which can help characterise bacteria, resolve them from electronic noise and debris, and indicate cell viability. Flow cytometry eliminates manual counting errors and allows simultaneous quantitative assessment of multiple cellular parameters at the single cell level within minutes of water sampling.
To differentiate signals from inorganic and organic particles and cell particles, the use of fluorescent dyes binding to nucleic acids are used.
To separate live and dead cells, several dyes can be used together. These dyes should have a similar excitation wavelength but different emission wavelengths. This will allow the dyes to be detected by separate fluorescence channels.
This method uses the BacLight live/dead system, which is a combination of SYBR™ green 1 and propridium iodide dyes.
SYBR™ green 1 passively enters both live and dead cells and fluoresces green. It binds to double stranded DNA.
Propridium iodide cannot cross intact bacterial membranes and therefore only enters membrane compromised cells. It has an intense red fluorescence and in part displaces already bound green dye and results in a dominant red colour.
Dead cells with compromised membranes appear red and live cells appear green.
The AccuriTM C6 has a pre-loaded software analysis template developed by researchers at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) to be used that has the gating set up.

Interferences:
Chlorine and chloramines: Neutralise by adding sodium thiosulphate which at a concentration of 18 mg/l should counteract up to 5 mg/l of free and combined residual chlorine.

Performance of Method:
Range of Application:

Results are expressed as live cells per ml and total cells per ml

Limit of Detection,

The limit of detection has not been calculated for this method

Uncertainty of measurement:

The uncertainty has not been calculated for this method

References:
Application of Flow Cytometry to YW Treatment Systems – DJ Baldock 2013
Assessing Water Quality with the BD Accuri™ C6 Flow Cytometer - Erin Gatza, Frederik Hammes,and Emmanuelle Prest 2013
BD Accuri™ C6 Software User Guide – 7820095-01 Rev-0