METHOD STATEMENT

Determinand:
Flow Cytometry

Matrix:
Sample Type: drinking, ground and surface waters

Principle of Method:
The flow cytometer uses hydrodynamic focusing to focus liquid samples into a single stream. A sheath fluid, in this case distilled water, is used to create a pressure differential and squeezes the cells into alignment.
The single stream of cells is taken up by the flow cytometer and is exposed to laser light.
The Partec SL has one laser emitting light of 488nm (blue).
The laser light passing the sample is recorded by a series of detection channels.
The data collected is:

a) Each particle passing through is recorded as a dot in a dot plot diagram.
b) Particle size is determined by forward scatter (FSC), which detects how much light is occluded by the particle passing the laser.
c) Particle complexity is determined by side scatter (SSC), the more complex a particle, the greater the amount of light scattering.

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 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.
Flow cytometry displays data in two different formats, dot plot and histograms. Dot plots are used for this method.
A dot plot displays every event recorded by the instrument as an individual dot on the graph. The axis chosen for the graph for this method are FL1 (green) against FL3 (red). The fluorescence axes are logarithmic, this allows for better distinction between fluorescent clusters. Regions of interest can be separated by digital gates, the contents of which can be counted and analysed.
The digital gates for this method have been set on the Partec SL and are not to be altered.

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 dead 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
Guide to using the Flow Cytometer – DJ Baldock 2012