METHOD STATEMENT

Determinand:
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. 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.
The Attune™ Acoustic Focusing Cytometer is an acoustic cytometer that uses acoustic radiation pressure to confine the cells or particles in a sample solution into a single, focused line along the central axis of the flow channel. The focusing is independent of the sample fluid flow and does not use high fluid velocities or high volumetric sheath fluid, which can damage cells.
The acoustically driven capillary is an acoustic resonant device that focuses the cells or particles into a single tight line using a capillary coupled to a single piezoelectric transducer. The acoustic contrast of a particle or cell is determined by the density and compressibility differences between the particle/cell and its background medium. The relative magnitudes and signs of the differences determine the size and direction of the acoustic radiation pressure force that is applied to the particles, which allows the particles traveling through the capillary to experience a time averaged force that aligns them along the central line of the capillary independent of fluid flow.
Like a conventional flow cytometer, the Attune™ Acoustic Focusing Cytometer includes three main systems: optics, fluidics, and electronics.
The optical system includes: an optical cell that is integrated with a laser, which acts as the light source for scatter and fluorescence; the detectors, which receive the light to illuminate the particles in the focused stream; and optical filters to direct the resulting light signals to the appropriate detectors.
The fluidics system uses a syringe pump that pushes or pulls the sample through the system. Fluid flow can be controlled at any desired rate using appropriate pumps.
The electronics system converts the detected light signals into electronic signals that can be processed by the computer system, which converts the signals from the detectors into digital data to perform the necessary analyses.
An important difference between the acoustically focused cytometer and a conventional hydrodynamically sheath–focused cytometer is that particles or cells in the acoustic capillary are focused toward a tight central line along the axis of the capillary whether fluid is flowing or not.
To differentiate signals from inorganic and organic particles and cell particles, the use of fluorescent dyes binding to nucleic acids are used.
To separate Total and Intact 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 and lasers.
This method uses the BacLight live/dead system, which is a combination of SYBR™ green 1 and propidium iodide dyes.
SYBR™ green 1 passively enters all cells and fluoresces green. It binds to double stranded DNA and is detected by the Blue laser.
Propidium 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. It is detected by the Yellow laser.
Cells with compromised membranes appear red and intact cells appear green.

Sampling and Sample Preparation:

Once taken, microbiological samples should be transferred immediately to dark storage conditions and kept at a temperature between 5 ± 3°C for transport to the laboratory. If samples are not analysed immediately on receipt in the laboratory, they should be kept at a temperature between 5 ± 3 °C, in dark conditions until analysis commences.
Samples should be analysed as soon as practicable. This is ideally on the day of collection however storage at the conditions noted in 6.1 for up to 80 hours from sampling has been shown to be suitable.
Where samples have been analysed beyond 80 hours from sampling, the customer should be informed or a statement reflecting this should be included with the report.

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 intact cell count per ml and total cell count per ml.
The LIMS system calculates the non-intact cell count per ml by subtracting intact cell count from total cell count.
Any dilution carried out on discoloured samples must be factored into the final result before it is entered onto the LIMS system.

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
Attune™ NxT Flow Cytometer Basic Training- Revision 2.4
Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide - Revision B.0
Attune™ Acoustic Focusing Cytometer Guide
Attune™ NxT Software User Guide- Revision F.0