METHOD STATEMENT

Determinand:
Pseudomonas aeruginosa

Matrix:
Raw and Potable waters

Principle of Method:
A known volume of the water sample is filtered through a membrane filter with 0.45µm pores upon which the bacteria are retained. The filter is then placed on a selective growth medium and incubated at 37°C for 40 - 48 hours, after which colonies characteristic of *Pseudomonas aeruginosa* are counted and picked off for confirmation.

Confirmation is carried out by inoculating Milk agar and examining for growth, pigment production and casein hydrolysis (clearing of the milk medium around the colonies). The confirmatory tests do not take account of non-pigmented strains of *Pseudomonas aeruginosa.*

As an alternative to traditional confirmation, colonies may be identified directly using a MALDI-TOF MS system to perform protein profiling.

Sampling and sample preparation:
Once taken, microbiological samples should be transferred immediately to dark storage conditions and kept at a temperature between 2 - 8°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 2 - 8°C, in dark conditions until analysis commences.
Samples should be analysed as soon as practicable on the day of collection. In exceptional circumstances, if there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.

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 (The Microbiology of Drinking Water 2002).
Plasticisers can interfere in the mass spectrometry analysis. Only certified, non-plasticising plastic materials should be used.

Performance of Method:

Range of Application:	0 - 100 cfu/100ml without dilution
Limit of Detection:	1 cfu/100ml
Normal Reporting Level:	0 cfu/100ml = Not Detected

References:
Environment Agency - The Microbiology of Drinking Water (2002) - Part 8 - Methods for the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa* by membrane filtration.