# METHOD STATEMENT

**Determinand:**
Pseudomonas aeruginosa

**Matrix:**
Sample Type: 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 entrapped. The filter is then placed on selective growth medium Pseudomonas agar supplemented with C-N 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 Cetrimide 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.
Where an exceedance has occurred the customer should be informed or a statement reflecting this should be included with the report (except where the customer has been already made aware that this is occurring on a regular basis and requests this not to be applied).

**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.
Process waters and pure waters may contain different biocides and the use of sodium thiosulphate may not be appropriate under these conditions. Customers should provide guidance when obtaining quotes.

**Performance of Method:**

**Limit of Detection:**
The Limit of Detection for this method is calculated as detailed in internal Procedure GOP7.2B.

**Uncertainty of measurement:**
The Uncertainty of Measurement for this method is calculated as detailed in internal Procedure GOP7.6C and the results are recorded on GQF7.6.3.

**References:**

Environment Agency - The Microbiology of Drinking Water (2010) - Part 8 - Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration.
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UNIPATH LTD, (1990). The Oxoid Manual, 6th Edition, Unipath Ltd., Basingstoke, UK.
Collins and Lyne's Microbiological Methods. Sixth Edition 1989. Page 133, Membrane Filter Counts.
Environment Agency - The Microbiology of Drinking Water (2002) - Part 2 - Practices and Procedures for Sampling.
Comparison of media for the confirmation of Pseudomonas aeruginosa colonies. Severn Trent Water, Quality Assurance. Wanlip Laboratory. Report No W21. January 1993.
Health Technical Memorandum 04-01: Safe Water In Healthcare Premises. Part B: Operational Management (2016)
International Organization for Standardization – ISO16266:2006 Water Quality – Detection and Enumeration of Pseudomonas aerugnionsa – Method by Membrane Filtration