

## METHOD STATEMENT

### **Determinand:**

Total viable count

### **Matrix:**

Clean waters where testing to EP or USP standards is required.

### **Principle of method:**

A viable count is used to determine the number of cells in the sample capable of forming colonies on a suitable agar medium, with the assumption that each viable cell can yield one colony. For this reason, the viable count is often called the colony count or plate count.

There are three ways of performing a colony count: the spread plate method, pour plate method and membrane filtration. Pour plates are prepared by pipetting a known volume of sample water into a sterile Petri dish and then adding molten agar and mixing gently by swirling the plate.

Spread plates are prepared by pipetting a volume of sample onto the surface of the agar. With membrane filtration, a volume of sample is filtered through a membrane filter. The filter is placed onto the surface of the agar plate. Following incubation under the relevant conditions, the numbers of colony forming units are counted.

### **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 are neutralised by adding sodium thiosulphate, which at a concentration of 18mg/l should counteract up to 5mg/l of free and combined residual chlorine.

### **Performance of method:**

Range of Application: 0 - 100 cfu/100ml or cfu/1ml  
Limit of Detection: 1 cfu/100ml or cfu/1ml  
Normal Reporting Level: 0 cfu/100ml or cfu/1ml = Not Detected

### **References:**

Environment Agency – The Microbiology of Drinking Water (2002) – Part 7 – Methods for the Enumeration of Heterotrophic Bacteria by pour and spread plate techniques.

Environment Agency – The Microbiology of Drinking Water (2002) – Part 3 - Practices and procedures for laboratories.

The European Pharmacopoeia 2008.

The United States Pharmacopeia 2008

NHS estates – washer-disinfectors validation and verification – Health Technical Memorandum 2030.

