## Test Procedures

## Total Bacteria Count

|  | Order Code |
| :--- | :--- |
| Bacteria Plates (40) | 778413 |
| Dilution Pots (40) | 77814 |
| 1 ml Sterile Syringes (30) | 778483 (3 packs required) |

This test is used for the quantification of total aerobic bacteria in water.
The sample is added to dehydrated culture media and incubated for 48 hours at $35^{\circ} \mathrm{C}+/-2^{\circ} \mathrm{C}$ (AOAC certified method). Aerobic bacteria show as red spots on the culture media and are counted to obtain the colony count as Colony Forming Units/ml (CFU/ml).

## Storage

Store bacteria plates between $1^{\circ} \mathrm{C}$ and $30^{\circ} \mathrm{C}$ and observe the expiry dates on the packaging. Store dilution pots between $1^{\circ} \mathrm{C}$ and $30^{\circ} \mathrm{C}$ and observe the expiry dates on the packaging.

## Sample Preparation

The collection bags supplied should be used to collect samples for total bacteria plate testing. The same sample can be used for this and the Coliform/E.Coli test. The sample bag contains a sodium thiosulphate tablet to remove any chlorine present in the sample. However, a clean sterile collection vessel of any type may be used as the total bacteria plate contains a chlorine neutralising agent.

## Test Range: 0-2000 CFU/ml

## Procedure

1) Take one of the sterile, 100 ml sample bags and collect 100 ml of water to be tested. Use the pictorial guide for proper sampling procedure with the thio bags. The same sample can be used for the E.Coli/Coliform test.

Note: It is important that the sample point is free from potential contamination. If necessary clean the sample point thoroughly prior to sampling. The sample bag contains a tablet to neutralise any chlorine that may be present in the sample. Allow this tablet to dissolve before continuing to step 2.
2) Have the following items ready for use:
$1 \times 100 \mathrm{ml}$ sample bag containing the sample to be tested
$2 \times$ new, sealed 1 ml syringes
1 x sterile alcohol wipe
$1 \times 9 \mathrm{ml}$ dilution pot
$1 \times$ TVC bacteria plate
The incubator should be switched on and allowed to reach $35^{\circ} \mathrm{C}$.
3) Wipe the foil surface of the dilution pot with the alcohol wipe and set the pot to one side (discard the wipe afterwards).
4) Using a new sterile 1 ml syringe, draw up exactly 1 ml of sample water from the sample bag.

Take care not to touch the inside of the bag and reseal immediately after sampling. The remaining sample can be used for the Coliform/E.Coli test.
5) Break the foil seal of the dilution pot with the base of the 1 ml syringe and transfer the 1 ml of sample into the pot. Remove the syringe and discard.

Swirl the pot gently to mix.
6) Take a new sterile 1 ml syringe and fill with exactly 1 ml of sample water from the dilution pot.
7) Open the lid of the TVC bacteria plate and slowly transfer the sample to the centre of the plate.
8) Cap the plate and transfer to the incubator.
9) Incubate at $35^{\circ} \mathrm{C}$ for 48 hours.
10) After 48 hours, place the plate on a white surface (or piece of paper) and count the number of red spot bacteria colonies on the surface of the plate.
11) The Colony Count (as CFU/ml) $=$ Number of red spot colonies counted x 10 . e.g. If 25 colonies are counted the final colony count for reporting purposes is $250 \mathrm{CFU} / \mathrm{ml}$. Ideally, total bacteria levels in potable water should be less than 100 CFU/ml.

## Guidance Notes for Plate Counting

If the water sample is highly contaminated (> 200 growing colonies), the plate will not be able to show single colonies. The amount of chromogenic substrate in the media is not enough to colour every colony. The plate will normally turn a complete pale pink colour, not showing any individual colonies. In this case, report the result as $>2000 \mathrm{CFU} / \mathrm{ml}$.

Sometimes samples with a huge bacteria load, way in excess of 200 growing colonies will result in an even white covered plate. This plate looks empty however it is fully covered with bacteria. Study the plate carefully to check for complete coverage.

Some bacteria such as Staphylococcus will produce different coloured growth on the plate (not red). These should be included in the plate count.

