

<b>Department</b>	<b>Micro Laboratory</b>	<b>Document no</b>	<b>MICLAB – METHOD 033</b>
<b>Title</b>	<b>TOTAL MICROBIAL PLATE COUNT</b>		
Prepared by:		Date:	Supersedes:
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## 1. **SCOPE**

This general test method shall be followed in order to determine the total viable bacterial count (Total Plate Count). This procedure applies to all finished products, raw materials, preservative efficacy and environmental testing.

Note: Total Plate Count also refers to the following terms: Total Aerobic Plate Count, Total Aerobic Viable Count and Standard Plate Count.

## 2. **REAGENTS AND MATERIALS REQUIRED**

- 2.1 Tryptone Soya Broth plus 4% Tween 80 (TSB + T) or other neutralising broth as predetermined by validation study.
- 2.2 Tryptone Soya Agar (TSA) or other as predetermined by validation study.
- 2.3 Sterile petri dishes
- 2.4 Sterile pipettes
- 2.5 Sterile stomacher bag
- 2.6 Filter sterilised 2,3,5 Triphenyl Tetrazolium Chloride 0.5% (TTC)

## 3. **GENERAL TEST METHOD**

- 3.1 Weigh out 10g of sample into the stomacher bag.
- 3.2 Make up to 100g with TSB + T (or other neutralising broth).
- 3.3 Homogenise well by placing it in the stomacher for 15-30 seconds or shake well if weighed directly into a bottle. This is a 1 in 10 dilution.
- 3.4 Plate out in duplicate 1ml and 0.1ml of the 1 in 10 dilution.

NOTE: One dilution only is permitted for validated samples where the recovery sensitivity is less than 10 (ie: -1 dilution).

NOTE: If the test method sensitivity is less than 100, then one dilution must be plated out (ie: -2 dilution).

- 3.5 Pour plates with approximately 15-20 mls of molten and tempered TSA kept in the water bath (45 - 48°C) - mix gently but thoroughly.

NOTE: Where sample is coarse, grainy or opaque and distinguishing between product and colonies would be difficult, add 1mL of TTC per 100mL TSA

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before pouring. The TTC will improve differentiation i.e. colonies stain red/pink.

- 3.6 Allow agar to solidify.
- 3.7 Incubate plates in an inverted position at  $30 \pm 1^\circ\text{C}$  for 5 days (or  $30^\circ\text{C} \pm 1^\circ\text{C}/3\text{days}$  for swabs and preservatives). Plates should be stacked no more than six high.
- 3.8 Remove plates and count colonies on plates that yield between 25 and 250 colony forming units (cfu) per plate.

**NOTE:** If the number of colonies per plate is more than 250, the result should be reported as greater than 250 times the dilution factor. For counts less than 25, the actual number of colonies on the lowest dilution is known as the estimated count of colony forming units (cfu) per gram and should be reported as the actual number of cfu's per gram.

- 3.9 Multiply the count by the inverse of the dilution factor.
- 3.10 Report all results to two significant figures.

Eg:        1340 =         $1.3 \times 10^3$         or 1300  
              1350 =         $1.4 \times 10^3$         or 1400  
              1370 =         $1.4 \times 10^3$         or 1400

Results for water testing are not recorded in scientific notation.

- 3.11 Report results as total plate count per gram of sample (or per mL for water or liquid samples).
- 3.12 If a high bacterial count is expected, in addition to the above test dilution, prepare serial dilutions by adding 1ml of initial dilution into 9ml of sterile 0.1% peptone diluent. Plate out dilutions as required using the technique described above.
- 3.13 All colonies grown must be fully identified according to QO-S00103 – Identification of Contaminants.