

Total Aerobic Plate Counts (Based on the BS EN ISO 4833:2003 Standard Protocol) **(for carcass swabs)**

MATERIALS

1. Reagents

- 1.1 Maximum recovery diluent (MRD) in 9 ml and 90 ml volumes.
- 1.2 Plate count agar (TBC) 15 ml per plate for food samples.

PROCEDURES

1. Perform decimal dilutions

- 1.1 Using a fresh sterile pipette transfer 1ml of the initial inoculum into 9 ml of MRD. Repeat the procedure for as many decimal dilutions as required.
- 1.2 Mix the dilutions using a vortex mixer for 5 to 10 seconds.

2. Inoculation

- 2.1 Aseptically inoculate 1 ml of the initial inoculum and each required dilution into a labelled Petri dish. Full compliance with BS EN ISO 4833:2003 requires duplicate plates to be used for each dilution.

3. Melting agar and pouring plates

- 3.1 A boiling bath will melt a 500ml pre-sterilised bottle of Plate Count Agar (PCA) in 60-90 minutes. Bottles should be warmed slightly before placing in very hot water (to avoid cracking). Do not overheat agar.
- 3.2 After melting, leave the agar on the bench for 20 minutes to ensure the bottle will not crack and then place in a water bath of temperature $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$. A single bottle will take roughly 1 hour to cool to 46°C .
- 3.3 The figures above are intended as a guide. Technicians should not rely entirely on these timings. The temperature of the agar bath should be checked before the first set of plates is poured. Agar should always be checked before use to ensure it has adequately melted and at the correct temperature.
- 3.4 Add 15 ml of tempered ($46 \pm 1^{\circ}\text{C}$) PCA agar to each Petri dish. Carefully mix by swirling six times clockwise, six times left to right, six times anticlockwise and six times up and down. Allow to set. The time elapsing between the preparation of the initial suspension and contact with the agar should not exceed 45 minutes.

4. Incubation

Invert the Petri dishes and place in stacks of six or less. Transfer to an incubator at $30 \pm 1^{\circ}\text{C}$ for 72 hours \pm 3 hours. If the incubator is fan-assisted, avoid using the shelf closest to the fan as this will dry the plates excessively.

5. Counting of colonies

- 5.1 Count all plates containing up to 300 colonies. Retain dishes containing not more than 300 colonies at two consecutive dilutions. At least one of these dishes must contain 15 colonies.
- 5.2 Examine the dishes under subdued light. It is important that pinpoint colonies should be included in the count, but it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies. If necessary use a zoom microscope to distinguish colonies from foreign matter.
- 5.3 Spreading colonies should be considered as single colonies. If less than one quarter of the dish is overgrown by spreading, count the colonies on the unaffected part of the dish and calculate the corresponding number of the entire dish. If more than one-quarter is overgrown by spreading colonies, discard the count.

6. Calculation

$$\text{Number of micro-organisms} = \frac{\Sigma c}{(n_1 + 0.1n_2) d}$$

- Σc = The sum of colonies counted
- n_1 = The number of dishes retained in the 1st dilution
- n_2 = The number of dishes retained in the 2nd dilution
- d = The dilution factor corresponding to the first dilution

Round the result calculated to two significant figures. Multiply the result by the total volume of diluent that was added to the swab and divide by the surface area of carcass that was sampled to determine the total aerobic count cm^{-2} .