Total Aerobic Plate Counts (Based on the BS EN ISO 4833:2003 Standard Protocol)
(for use with Minced Meat or Mechanically-Separated Meat)

MATERIALS

1. Reagents

1.1 Maximum recovery diluent (MRD) in 9ml, 90ml and 225ml volumes.
1.2 Plate count agar (TBC) 15 ml per plate for food samples.

SAMPLE PREPARATION

1.0 A 25g sub-sample of product is required for testing. It is envisaged that most of the samples that will be supplied to laboratories for testing will be retail packs of minced meat.

1.1 Unless the packaging or wrapping material is very thin and could be damaged by the cleaning process (e.g. some cling film-wrapped portions of meat on trays), or the package contents can be removed without any risk of contamination, the package should be cleaned and sanitised before opening.

1.2 Clean the external surface of rigid or semi-rigid packages of meat with detergent and water ensuring no contamination of the package contents occurs.

1.3 Dry the package with a clean towel then more thoroughly with clean, single-use absorbent paper.

1.4 Sanitise the packages over such a part of the exterior that contamination is avoided on opening with alcohol-soaked wipes. Sanitation should be carried out very carefully to prevent contamination of the package contents. Allow all alcohol to evaporate before opening the package.

1.5 Open the package of ground meat using sterile scalpels, scissors or forceps. All operations during and after opening shall be carried out under aseptic conditions preferably without interruption.

1.6 If required, generate a secondary sample for testing from the package contents. The testing sample should be obtained using sterile spoons and removing randomly-selected individual sub-samples, each of not more than 1g mass to produce an appropriate mass of sample for testing.

1.7 Add the test sample of minced meat or MSM to a stomacher bag containing 9 volumes of maximum recovery diluent (MRD) and stomach for 1 minute.

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°C ± 1°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 ± 1°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 ± 1°C for 72 hours ± 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

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

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. Report the result as the total aerobic count g^{-1} test material.