

Enumeration of β -glucuronidase-positive *Escherichia coli* from minced meat, mechanically-separated meat and meat products using poured plates (Based on the BS EN ISO 16649-2:2001 Standard Protocol)

Please note this SOP is based on the BS EN ISO 16649-2:2001 standard. If preferred, it is equally acceptable for laboratories to use the BS EN ISO 16649-1 standard which involves filtration of sample diluent followed by culture of *E. coli* on Tryptone bile glucuronic agar using nitrocellulose membranes.

MATERIALS

1. Reagents

- 1.1 Maximum recovery diluent (MRD) in 9ml, 90ml and 225ml volumes.
- 1.2 Tryptone bile glucuronic agar (TBX) 15ml per plate.

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 subsamples, each of not more than 1g mass to produce an appropriate mass of test aliquot.
- 1.7 Add the sample of minced meat to a stomacher bag containing 9 volumes of 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 16649-2:2001 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 Tryptone bile glucuronic agar (TBX) 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 15ml of tempered ($46 \pm 1^{\circ}\text{C}$) TBX 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 $37 \pm 1^{\circ}\text{C}$ for 4 hours before further incubation at $44 \pm 1^{\circ}\text{C}$ for 21 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 and count the β -glucuronidase-positive *Escherichia coli* colonies which are coloured blue.

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.

Express the results as the number of β -glucuronidase-positive *E. coli* g^{-1} of sample.