

Detection of *Salmonella* from minced meat, mechanically separated meat (MSM) and meat products (Based on the ISO 6579:2002 Standard Protocol)

MATERIALS

1. Reagents

- 1.1 Rappaport Vassiliadis Broth with soya (RVS) in 10 ml volumes.
- 1.2 Muller Kauffmann tetrathionate-novobiocin broth (MKT broth) in 10 ml volumes.
- 1.3 Pre - poured & dried plates of xylose lysine deoxycholate agar - XLDA (primary selective agar).
- 1.4 Pre - poured & dried Brilliant Green Agar plates (BGA).
- 1.5 Pre - poured & dried Nutrient Agar plates (NA).
- 1.6 Biochemical identification kit (e.g. API20E).
- 1.7 Poly O, Poly H & Poly Vi antisera.
- 1.8 Bacteriological saline 0.85%.
- 1.9 Buffered peptone water (BPW) in 90ml or 225ml volumes
- 2.0 Semi-solid nutrient (SSN) agar.

SAMPLE PREPARATION

- 1.0 Depending on the product and its intended usage, either a 10g or 25g sub-sample is required for testing. Food Business Operators will need to advise their laboratories as to the test quantity. 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 to a stomacher bag containing 9 volumes of buffered peptone water (BPW) and stomach for 1 minute.

SAMPLE POOLING

- 1.1 In order to save money with testing costs, sample pooling for minced meat, MSM and meat products is allowed. If samples are pooled for testing, the following ratio of sample and BPW should be used:

- For 10g test samples, 5 samples should be pooled together to generate a mass of 50g total. 2 volumes of BPW should be used for Pre Enrichment.
- For 25g test samples, 5 samples should be pooled together to generate a mass of 125g total. 5 volumes of BPW should be used for Pre Enrichment.

PROCEDURES

1. Pre- enrichment

- 1.1 Incubate the sample in BPW at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 to 20 hours.

2. Selective Enrichment

- 2.1 A - Transfer 0.1 ml of the pre-enriched sample into 10 ml of Rappaport Vassiliadis Broth with soya (RVS) medium. Incubate at $41.5 \pm 1^{\circ}\text{C}$ for 24 ± 3 hours.
- 2.2 B - Transfer 1 ml of the pre-enriched sample into 10 ml of Muller Kauffmann tetrathionate-novobiocin broth (MKT). Incubate at $37 \pm 1^{\circ}\text{C}$ for 24 ± 3 hours.

3. Plating Out

- 3.1 After 24 ± 3 hours, using the RVS culture, inoculate by means of a sterile loop two plates of xylose lysine deoxycholate agar (XLDA) so that well-isolated colonies are obtained. Do not recharge the loop when streaking the second plate. Repeat for the second selective agar using a fresh sterile loop.
- 3.2 After 24 ± 3 hours, using the MKT culture, inoculate XLDA and Brilliant Green Agar (BGA plates) as above.

4. Incubation

- 4.1 Invert the Petri dishes and transfer to an incubator at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 ± 3 hours.
- 4.2 Typical colonies of *Salmonella* grown on XLDA have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator.

- 4.3 *Salmonella* H₂S negative variants (e.g. *S. paratyphi* A) grown on XLDA are pink with a darker pink centre. Lactose-positive *Salmonella* grown on XLDA are yellow with or without blackening.
- 4.4 Typical colonies of *Salmonella* on BGA are pink, 1 mm to 2 mm in diameter, and cause the colour of medium to change to red.
- 4.5 Any typical or suspect colony should be confirmed. A slide agglutination test may be performed at this stage to aid selection of suspect colonies.

5. Confirmation

- 5.1 Take from each pair of plates of each selective medium at least one colony considered to be typical or suspect and a further four if the first is negative (i.e. 20 per sample maximum).
- 5.2 If there are fewer than five suitable colonies, take all the available colonies through confirmation.
- 5.3 Streak these colonies onto pre-dried Nutrient plates to obtain well isolated colonies. Incubate at $37 \pm 1^{\circ}\text{C}$ for 24 ± 3 hours, and use these cultures for confirmatory tests.

6. Serological confirmation (can be undertaken by an external reference laboratory)

Test for the presence of *Salmonella* antigens by slide agglutination with the appropriate sera, from pure colonies after auto-agglutinable stains have been eliminated.

This method relies on the antibody/antigen reaction between a test culture and commercially prepared antiserum.

6.1 Elimination of auto-agglutinable strains

- Place one drop of saline onto a clean glass slide.
- Disperse in this drop part of the colony to be tested or a colony from a pure culture, so as to obtain a homogenous and turbid suspension.
- Rock the slide gently for 30 → 60 seconds.
- Observe the result against a dark background, preferably with the aid of a magnifying glass. If the bacteria have clumped together into more or less distinct units, the strain is considered auto-agglutinable, and the detection of antigens will be impossible.
- In practice, auto - agglutinating strains of *Salmonella* are rare; it is more economical to perform poly O, H and Vi serology first.

6.2 Examination for O antigen

- Using one pure colony, recognised as non-autoagglutinable, proceed as above, using one drop of the anti O serum instead of saline solution.

- If agglutination occurs, the reaction is considered positive for the presence of that antigen.

6.3 Examination for H antigens

- Inoculate a Semi-Solid Nutrient Agar (SSNA) slope with a pure non-autoagglutinable colony from the XLDA or BGA plate. Incubate at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hours.
- Use this culture for examination for H antigens, proceeding as above, but using one drop of the anti H serum instead of saline solution.
- If agglutination occurs, the reaction is considered positive for the presence of H antigen.

6.4 Examination for Vi antigens

- Perform Vi serology as per either section 6.2 or 6.3 above.

7. Biochemical confirmation

Perform an oxidase test according to the manufacturer's instructions (*Salmonella* are oxidase negative).

On oxidase negative colonies, use an API20E biochemical test kit (or equivalent) following the Manufacturer's instructions. Only one API 20E need be used for each typical or suspect colony type. It is important when using the API 20E system that a pure culture has been used.

8. Results

Biochemical Reactions	Auto - agglutination	Serological reactions	Interpretation
Typical	No	O, Vi or H antigen positive	Confirmed <i>Salmonella</i>
Typical	No	All reactions negative	Presumptive <i>Salmonella</i>
Typical	Yes	Not tested	Presumptive <i>Salmonella</i>
Not typical	No	O, Vi or H antigen positive	Presumptive <i>Salmonella</i>
Not typical	No	All reactions negative	Not considered <i>Salmonella</i>

- 8.1 Samples which fall into the group 'confirmed *Salmonella*' need to be sent to one of the FSA-approved *Salmonella* reference centres for definitive typing.

9. Reporting

Report the presence or absence of *Salmonella* in the sample mass.