Detection of *Salmonella* for red meat samples (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 Semi-solid nutrient (SSN) agar.

PROCEDURES

1. Pre- enrichment

1.1 After removing a small volume of the BPW for total aerobic counts and the enumeration of *Enterobacteriaceae* incubate the remainder of the BPW and the sponge at 37°C ± 1°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 ± 1°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± 1°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°C ± 1°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* H_2S 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 ± 1°C for 24 ± 3 hours, and use these cultures for confirmatory tests.

6. Serological confirmation

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± 1°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 *Salmonella*
Typical	No	All reactions negative	Presumptive *Salmonella*
Typical	Yes	Not tested	Presumptive *Salmonella*
Not typical	No	O, Vi or H antigen positive	Presumptive *Salmonella*
Not typical	No	All reactions negative	Not considered *Salmonella*

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 result as present or absent in the area of the carcass sampled.