

# STANDARD OPERATING PROCEDURE (SOP)

## INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD organised by EURL-*Salmonella* FOOD STUDY V- 2011

### 1 Scope and field of application

This standard operating procedure (SOP) describes the procedure for the detection of *Salmonella* in the presence of competitive micro-organisms in a food matrix. For this purpose Reference Materials (RMs) containing *Salmonella* Typhimurium (STM) or *Salmonella* Enteritidis (SE), as prepared by the Health Protection Agency (HPA, United Kingdom), are used. As matrix, minced meat (negative for *Salmonella*) is used. The application of this SOP is limited to the interlaboratory comparison study for *Salmonella* described in this SOP.

### 2 References

International Standard – ISO 6579: 2002(E) Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

ISO 6579:2002/Amd 1 2007. Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faces and in environmental samples from the primary production stage.

Lenticule Disc Handling Information. HPA Culture Collection, Salisbury, United Kingdom. More information on the reference materials (lenticule discs) as produced by the HPA can be found on: <http://www.hpacultures.org.uk/products/lenticulediscs/index.jsp>

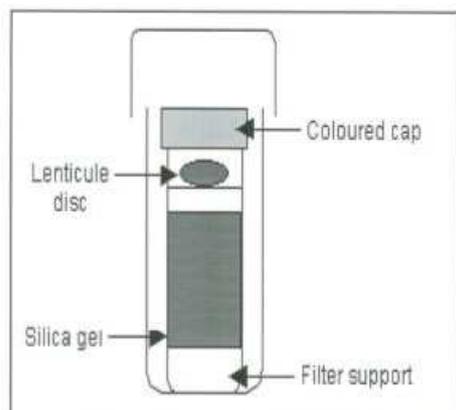
### 3 Definitions

For the purpose of this SOP, the following definitions apply:

-*Salmonella*: micro-organisms which form typical colonies on isolation media for *Salmonella* and which display the serological and/or biochemical reactions described when tests are carried out in accordance with this SOP.

-*Reference Material*: a lenticule disc containing microorganism at a defined number in a water soluble matrix.

Note: Each lenticule is individually packed in small vials as indicated in the picture below.



HPA, UK

#### 4 Principle

The detection of *Salmonella* involves the following stages:

- 1) Pre-enrichment
- 2) Selective enrichment
- 3) Isolation
- 4) Confirmation of typical colonies as *Salmonella*.

#### 5 List of abbreviations

BPW	Buffered Peptone Water
MKTTn	Muller Kauffmann Tetrathionate novobiocin broth
MSRV	Modified semi-solid Rappaport Vassiliadis medium
RM	Reference Material
RVS	Rappaport Vassiliadis medium with Soya
SOP	Standard Operating Procedure
XLD	Xylose Lysine Deoxycholate agar

#### 6 Methods and culture media

For this study the prescribed method is ISO 6579, with an extra incubation step of 24 h of the selective enrichment media. Additional to ISO 6579, it is requested also to apply Annex D of ISO 6579.

Non selective pre-enrichment medium	BPW (6.1)
Selective enrichment medium	MKTTn & RVS (prescribed)(6.2) MSRV (requested) (6.2)
Selective plating medium for first and second isolation	XLD and a second medium for choice (obligatory!)(6.3)
Confirmation media	see 6.4

Composition and preparation of the media and reagents are described in Annex B, and in Annex D of the ISO 6579: 2002. In the list of media given in 6.1 up to 6.4, reference is made to the relevant part of ISO 6579. Complete ready-to-use media or dehydrated media are also allowed to be used, as long as the composition is in accordance with the information given below. Check the quality of the media before use.

In addition to the prescribed method (ISO 6579) and requested method (Annex D of ISO 6579) it is possible to use other methods, e.g. the one(s) routinely used in your laboratory ['Own' method(s)]. This can vary from another culture method to 'a PCR technique'. If necessary prepare media for the 'own' method(s) according to the relevant instructions. Record all relevant information in the test report.

### **6.1 Non selective pre-enrichment medium**

-Buffered Peptone water (BPW) (ISO6579 Annex B.1)

### **6.2 Selective enrichment medium**

-Rappaport Vassiliadis medium with soya (RVS broth) (ISO6579 Annex B.2)

-Muller Kauffmann tetrathionate-novobiocin broth (MKTTn) (ISO6579 Annex B.3)

-Modified Semi solid Rappaport Vassiliadis (MSRV) (requested) (ISO6579 Annex D)

-Own selective enrichment medium used in your laboratory (optional)

### **6.3 Solid selective media for first and second isolation**

-Xylose-Lysine-Desoxycholate (140 mm and 90 mm plates) (ISO6579 Annex B.4)

-Second isolation medium for choice (obligatory)

-Own medium used in your laboratory (optional)

### **6.4 Confirmation media**

-Biochemical confirmation as described in ISO 6579 Annex B.6-B.11 or by reliable, commercially available identification kits.

-Nutrient agar (optional) (ISO6579 Annex B.5)

## **7 Apparatus and glassware**

The usual used microbiological laboratory equipment. If requested, note specifications of the apparatus and glassware on the test report.

### **7.1 Apparatus**

-Oven (for dry sterilisation) or autoclave (for wet sterilisation);

-Water bath or incubator, capable of operating at  $37\text{ °C} \pm 1\text{ °C}$  ;

-Water bath or incubator, capable of operating at  $41.5\text{ °C} \pm 1\text{ °C}$

-Sterile loops of 1  $\mu\text{l}$  and of 10  $\mu\text{l}$ ;

-pH-meter; having an accuracy of calibration of  $\pm 0.1\text{ pH unit}$  at  $25\text{ °C}$ .

### **7.2 Glassware**

-Sterile culture containers (bottles, jars or plastic bags) with nominal capacity of approximately 400 ml;

-Sterile culture bottles or jars with nominal capacity of 300 ml;

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- Sterile culture tubes with approximate sizes: 8 mm in diameter and 160 mm in length;
  - Sterile micro-pipettes; nominal capacity 0.1 ml and 1 ml;
  - Sterile petri dishes; standard size (diameter 90 mm to 100 mm) and/or large size (diameter 140 mm).

## 8 Procedure

Below the prescribed and the requested method of the interlaboratory comparison study in a food matrix of EURL-*Salmonella* is described. The different steps in the procedure are also summarized in Annex A of this SOP. In addition to these methods it is also allowed to use one or more own methods. Please record all relevant data in the test report. Details of the prescribed method can be found in ISO 6579. Details of the requested method can be found in Annex D of ISO 6579 (2007).

For testing the samples use as much as possible the materials you are normally using for your routine samples. For example, either use bags or jars for the pre-enrichment in BPW depending on what you routinely use. Bottles, bags or jars for the pre-enrichment in BPW are further mentioned as containers.

### 8.1 Pre-enrichment (day 1)

Use BPW equilibrated to at least room temperature (follow your routine procedure). Record in the test report (pages 2 & 3) the requested data on BPW.

Take the numbered vials with the *Salmonella* lenticules out of the freezer, 10-15 minutes before they are added to the BPW, to allow them to equilibrate to room temperature.

-Label 34 containers as follow:

- 25 containers from B1 to B25
- 9 containers from C1 to C9 (control lenticules)

-Add 25 g of meat to each container labelled B1 – B25 and C9.

-Add 225 ml BPW to each container (B1- B25 and C1-C9).

When your containers are already pre filled with 225 ml BPW, add 25 g of meat to the BPW.

**Add no matrix/ meat to the containers labelled C1 – C8.**

One container is a procedure control to which no lenticule or meat is added (= C8).

One container is the negative meat control to which only 25 g meat is added (= C9).

These control containers should be handled in the same way as the other containers.

-Add to the 32 labelled containers (containing BPW with or without meat) a lenticule disc from the vial with the corresponding label number (B1- B25 and C1 – C7).

**No lenticules are added to C8 and C9.**

- Leave all the containers for 10 – 15 minutes at room temperature to re-hydrate the lenticule. As the disc is coloured, it may be visible when it is re-hydrated. Even when it is not visible whether the lenticule is re-hydrated, proceed with the next steps of the procedure after 15 minutes standing at room temperature.

- Mix the samples by using a pulsifier or a stomacher. When this is not possible (eg when the samples are contained in a jar), shake the samples carefully.

-Incubate all samples at  $37\text{ °C} \pm 1\text{ °C}$  for  $18\text{ h} \pm 2\text{ h}$ . Record the temperature and time at the start and at the end of the incubation period and other requested data on page 3 of the test report.

If PCR is performed, record all requested data on pages 20-21 & 30 of the test report.

## 8.2 Selective enrichment (day 2)

Allow the selective enrichment broths RVS and MKTTn (prescribed method) to equilibrate to room temperature, if they were stored at a lower temperature. Dry the surface of the MSR/V plates (requested method), if necessary. Record (page 4-11) the requested data of the selective enrichment broths (RVS and MKTTn), MSR/V plates and own selective enrichment media (if used) in the test report.

-Label 34 RVS tubes, MKTTn tubes and MSR/V plates as follow:

- 25 tubes/plates from B1 to B25
- 9 tubes/plates from C1 to C9 (control)

If other selective enrichment media are used, label them in the same way as described above.

After equilibration:

### Prescribed methods:

-Transfer 0.1 ml of each BPW culture to each tube with a corresponding label containing 10 ml RVS medium. Incubate at  $41.5\text{ °C} \pm 1\text{ °C}$  for  $24\text{ h} \pm 3\text{ h}$  and later on for another  $24\text{ h} \pm 3\text{ h}$ ;

-Transfer 1 ml of each BPW culture to each tube with a corresponding label containing 10 ml MKTTn medium. Incubate at  $37\text{ °C} \pm 1\text{ °C}$  for  $24\text{ h} \pm 3\text{ h}$  and later on for another  $24\text{ h} \pm 3\text{ h}$ ;

### Requested method:

-Inoculate each MSR/V plate with three drops of each BPW culture with a corresponding label. Inoculate a MSR/V plate with a total volume of 0.1 ml. Incubate (**not upside down**) at  $41.5\text{ °C} \pm 1\text{ °C}$  for  $24\text{ h} \pm 3\text{ h}$  and if negative for another  $24\text{ h} \pm 3\text{ h}$ ;

### Optional method:

-Inoculate the routinely used selective medium/media (other than those mentioned above), with the corresponding BPW culture (note the inoculation volume of BPW used and the volume of the selective medium/media on the test report). Incubate at the temperature routinely used.

-Place the jars/tubes/plates in the appropriate incubator(s)/water bath(s) and record the temperature and time for the different enrichment media at the start and at the end of the incubation period and other requested data in the test report (page 4-11).

## 8.3 Isolation media (first and second isolation) (day 3 and 4)

Record in the test report (page 12-17) the requested data of the isolation media used.

Allow the isolation media to equilibrate to room temperature, if they were stored at a lower temperature. Dry the surface of the plates, if necessary.

-Label two times 34 large size Petri dishes and 34 standard size Petri dishes of each isolation medium from B1 to B25 and C1 to C9.

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*Note: In case you do not have large dishes (140 mm) at your disposal, use two standard size (90 -100 mm) dishes, one after the other, using the same loop.*

### **First isolation after 24 h**

#### *Inoculation:*

-Inoculate, by means of a 10 µl loop, from MKTTn and RVS cultures the surface of isolation media in large size Petri dishes (or two standard size Petri dishes) with the corresponding label numbers. Inoculate, by means of a 1 µl loop from suspect MSRV plates, the surface of isolation media in one standard size Petri dish with the corresponding label numbers. Inoculate the isolation media in such a way that isolated colonies will be obtained.

The following isolation media will be used:

- 1) Xylose Lysine Desoxycholate agar (XLD)  
Incubate the inoculated plates (with the bottom up) at  $37\text{ °C} \pm 1\text{ °C}$ . Record temperature and time and other requested data in the test report, page 12-13.
- 2) Second isolation medium. Follow the instructions of the manufacturer. Record temperature and time and other requested data in the test report, page 14-15.
- 3) Optionally: selective isolation medium/media routinely used in your laboratory.  
Incubate the medium/media at the temperature routinely used and record temperature and time and other requested data in the test report, page 16-17.

After incubation for  $24\text{ h} \pm 3\text{ h}$ , examine the Petri dishes for the presence of typical colonies of *Salmonella*.

### **Second isolation after 48 h**

After a total incubation time of  $48\text{ h} \pm 3\text{ h}$  of the selective enrichment media, repeat the procedure described above (**First isolation after 24 h**). Repeat the full procedure only when the First isolation after 24 h on selective enrichment media is negative.

## **8.4 Confirmation of colonies from first and second isolation (day 4 and day 5)**

For confirmation, take from each Petri dish of each selective medium, at least 1 colony considered to be typical or suspect (use only well isolated colonies). Store the plates at  $5\text{ °C} \pm 3\text{ °C}$ .

Before confirmation (see below), optionally, streak the typical colonies onto the surface of nutrient agar plates with the corresponding label numbers, in a manner which allows to develop well isolated colonies. Record on the test report (page 18) the requested data of the nutrient agar. Incubate the inoculated plates at  $37\text{ °C} \pm 1\text{ °C}$  for  $24\text{ h} \pm 3\text{ h}$ .

If the selected colony is not confirmed as *Salmonella*, test at maximum another 5 typical colonies from the original isolation medium (stored at  $5\text{ °C}$ ). Report the number of colonies tested and the number of colonies confirmed as *Salmonella* for each dish in Table 1 (isolation from RVS), Table 2 (isolation from MKTTn), Table 3 (isolation from MSRV) and Table 4 (isolation from own selective enrichment medium) on the test report pages 22-29.

For the results of detection of *Salmonella* using PCR complete in Table 5 on the test report at page 30.

**Confirmation of identity**

The identity from the colony selected above (either directly from the isolation medium, or from nutrient agar) is confirmed by means of appropriate biochemical and serological tests. Follow the instructions of ISO 6579. Note in the test report (page 19) which media/tests have been used for confirmation. The interpretation of the biochemical tests is given in Table 1 of ISO 6579:2002 on page 9. Optionally inoculate other media which are routinely used for confirmation. Record in the test report (page 19) the requested data.

**Conserve one positive isolate (*Salmonella* strain) from each sample.**

After the interlaboratory comparison study it may be necessary to perform some additional testing (in case of deviating results). Therefore it is requested to conserve one *Salmonella* confirmed colony from one of the used isolation media of each of the used selective enrichment medium from each suspect sample B1-B25 and C1-C9.

**9 Test report**

Record in the test report all information that might influence the results and is not mentioned in this SOP. Also record incidents or deviations from the specified procedures. The test report should include the name of the person in charge for the NRL, and the names of the persons who are carrying out the work. If the study was carried out by another laboratory than the NRL, please also give the details of this laboratory in the test report.

<b>Scheme of Bacteriological Interlaboratory Comparison Study FOOD V (2011) On detection of <i>Salmonella</i> spp. in minced meat (see Annex A)</b>		
Day	Topic	Description
1	<b>Pre-enrichment</b>	Allow the BPW to equilibrate to at least room temperature Add 25 g meat to container (jar or plastic bag) Add 225 ml BPW to meat (or add 25 meat directly to 225 ml BPW) Add 1 lenticule disc to BPW Leave 10- 15 minutes at room temperature Mix (pulsifier or stomacher) sample Incubate (18 h ± 2) h at (37 °C ± 1) °C
2	<b>Selective enrichment</b>	0.1 ml BPW culture in 10 ml RVS, incubate at (41.5 ± 1) °C for (24 ± 3) h 1 ml BPW culture in 10 ml MKTTn, incubate at (37 ± 1) °C for (24 ± 3) h 0.1 ml BPW culture on MSR/V plate, incubate at (41.5 ± 1) °C for (24 ± 3) h Own selective enrichment medi(um)(a)
3	<b>First isolation after 24 h</b>	Inoculate from RVS, MKTTn, suspect MSR/V plates (24h) and own medi(um)(a): <ul style="list-style-type: none"> <li>• Xylose Lysine Desoxycholate agar, incubate at (37 ± 1) °C for (24 ± 3) h</li> <li>• Second isolation medium* (obligatory)</li> <li>• Own selective medi(um)(a)* (optional)</li> </ul> *= incubate for specified time at the specified temperature
3	<b>Continue selective Enrichment</b>	Incubate RVS, MKTTn, MSR/V and own medium another 24 (± 3) hours at the relevant temperatures
4	<b>Second isolation after 48 h</b>	If the first isolation was negative, inoculate from RVS, MKTTn, suspect MSR/V plates (48 h) and Own medi(um)(a): <ul style="list-style-type: none"> <li>• Xylose Lysine Desoxycholate agar, incubate at (37 ± 1) °C for (24 ± 3) h</li> <li>• Second isolation medium*</li> <li>• Own selective medi(um)(a)*</li> </ul> * = incubate for specified time at the specified temperature
4	<b>Confirmation of identity</b>	Confirm the identity of the <i>Salmonella</i> suspect colonies from the first isolation media (day 3).
5	<b>Confirmation of identity</b>	Confirm the identity of the <i>Salmonella</i> suspect colonies from the first isolation media (day 4).

Annex A of SOP

**Interlaboratory comparison study food V  
on the detection of *Salmonella* spp. in minced meat  
EURL-*Salmonella* 2011**

