
STANDARD OPERATING PROCEDURE (SOP) INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD SAMPLES organised by EURL-*Salmonella* STUDY VI - 2013

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 sample. The matrix is minced chicken meat. 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 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.

3 Definitions

For the purpose of this SOP, the following definition applies:

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.

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
RVS	Rappaport Vassiliadis medium with Soya
SOP	Standard Operating Procedure
XLD	Xylose Lysine Deoxycholate agar

6 Culture media

For this study the prescribed method is ISO 6579, additional it is requested also to apply Annex D of ISO 6579. The following media are needed.

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

Composition and preparation of the media and reagents are described in Annex B, and in Annex D of ISO 6579: 2002. In the list of media given in 6.1 up to 6.4, reference is made to the relevant parts 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 and requested method (ISO 6579 and Annex D of ISO 6579) laboratories can perform an own method like PCR.

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)

6.3 Solid selective media for first and second isolation

- Xylose-Lysine-Desoxycholate (XLD) agar (90 mm plates) (ISO6579 Annex B.4)
- Second isolation medium of choice (obligatory)

6.4 Confirmation media

- Biochemical and/or serological 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 microbiological laboratory equipment. Note specifications of the apparatus and glassware.

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 $1\text{ }\mu\text{l}$ and of $10\text{ }\mu\text{l}$;
- pH-meter; having an accuracy of calibration of $\pm 0.1\text{ pH unit}$ at 25 °C .

7.2 Glassware

- 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 requested method of the sixth interlaboratory comparison study in food samples (minced chicken meat) of EURL-*Salmonella* is described. The different steps in the procedure are also summarized in Annex A of this SOP. Please record all relevant data. Details of the method can be found in ISO 6579 and Annex D of ISO 6579. For testing the samples, use as much as possible the materials you are normally using for your routine samples.

We ask you to perform the controls (especially the positive control) your laboratory is normally using for analysing routine samples for the detection of *Salmonella*. Three plastic bags numbered C1 to C3 are included in your parcel (C2 contains 25 g of minced chicken meat, C1 and C3 are empty).

We ask you to perform the following controls:

- C1: BPW only
- C2 :minced chicken meat (to which BPW need to be added)
- C3 : positive control as routinely performed in your laboratory (e.g.a reference material or a (diluted) culture containing *Salmonella*) to which BPW is added.

Record the requested data of the three control samples through the web based reporting form.

8.1 Pre-enrichment (day 1, Monday 30 September 2013)

Use BPW equilibrated to at least room temperature (follow your routine procedure).

Record the requested data of BPW.

Take the numbered plastic bags ('Whirl-pak').

Add 225 ml BPW to each plastic 'Whirl-pak' bag (B1- B18 and C1-C3).

Add your own positive control to plastic 'Whirl-pak' bag C3 or mark your positive control as C3.

Before proceeding, ensure that the minced chicken meat is at the bottom of the plastic 'Whirl-pak' bag immersed completely in the BPW. If necessary, knead the bag to immerse the meat completely.

Mix the samples by using a pulsifier, stomacher (rotary blender) or 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.

8.2 Selective enrichment (day 2, Tuesday 1 October 2013)

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 the requested data of the selective enrichment broths (RVS and MKTTn) and MSR/V plates.

-Label 21 RVS tubes, MKTTn tubes and MSR/V plates as follow:

- 18 tubes/plates from B1 to B18
- 3 tubes/plates from C1 to C3 (control)

After equilibration:

Prescribed methods:

- Transfer 0.1 ml of each BPW culture to each tube containing 10 ml RVS medium. Incubate at $41.5\text{ °C} \pm 1\text{ °C}$ for $24\text{ h} \pm 3\text{ h}$.
- Transfer 1 ml of each BPW culture to each tube containing 10 ml MKTTn medium. Incubate at $37\text{ °C} \pm 1\text{ °C}$ for $24\text{ h} \pm 3\text{ h}$.

Requested method:

- Inoculate the MSR/V plates with three drops of each BPW culture, with a 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 $24\text{ h} \pm 3\text{ h}$.

Record the temperature and time at the start and at the end of the incubation period and other requested data.

8.3 Isolation media (first and second isolation, day 3 and 4, Wednesday 2 October and Thursday 3 October 2013)

Record the requested data of the isolation media used.

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 MSR/V 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:

Inoculation:

Inoculate from suspect MSR/V plates, the surface of an isolation medium in one standard size Petri dish with the corresponding label number in such a way that well isolated colonies will be obtained. The following isolation media have to be used:

- 1) Xylose Lysine Desoxycholate agar (XLD)
Place the Petri dishes with the bottom up in the incubator set at 37 °C ± 1 °C (for 24 h ± 3 h).
- 2) Second isolation medium. Follow the instructions of the manufacturer.

Record temperature and time and other requested data of the isolation media.

After incubation for 24 h ± 3 h, examine the isolation media for the presence of typical or suspect colonies of *Salmonella*.

Second isolation after 48 h

After a total incubation time of 48 h ± 3 h of the MSR/V repeat the procedure described above (**First isolation after 24 h**). Repeat the full procedure only when the First isolation after 24 h of MSR/V is negative.

8.4 Confirmation of colonies from first and second isolation (day 4 and day 5, Thursday 3 October and Friday 4 October 2013)

For confirmation take from each Petri dish of each isolation medium at least 1 colony considered to be typical or suspect (use only well isolated colonies). Store the plates at 5 °C ± 3 °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. Incubate the inoculated plates at 37 °C ± 1 °C for 24 h ± 3 h.

If the selected colony is not confirmed as *Salmonella*, test a further 4 typical colonies from the original isolation media (stored at 5 °C). Record the results for each dish.

Table 1: isolation from RVS

Table 2: isolation from MKTTn

Table 3: isolation from MSR/V

For the results of detection of *Salmonella* when using PCR, complete Table 4.

Confirmation of identity

The identity from the colonies selected above (either direct from the isolation medium, or from nutrient agar) is confirmed by means of appropriate biochemical and serological tests. Follow the instructions of ISO 6579. Record which media and tests have been used for confirmation.

Store 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 store at least one *Salmonella* confirmed colony from one of the used isolation media of each used selective enrichment media from each suspect sample B1-B18 and C1-C3.

9 Reporting of the results

This is the first food EURL-*Salmonella* detection study for which all data have to be reported through an electronic reporting form. The web based report should contain all information that might influence the results and is not mentioned in this SOP. It is no longer necessary to report results of 'own' culture media. Results of an 'own' PCR method can still be recorded. Incidents or deviations from the specified procedures should also be recorded at the end of the test report (remarks/ comments). Furthermore, the name of the person in charge of the NRL, and the name(s) of the person(s) carrying out the work are requested to be recorded. If the study was carried out by another laboratory than the NRL, please also give the details of this laboratory.

We have made a selection of the most important questions in the web based test report, resulting in a shorter test report than before. As all NRLs are accredited according to ISO 17025, we assume that additional information, not requested in the test report, will be available in your system. In case of deviating results, the EURL-*Salmonella* may ask the NRLs to send additional information.

Overview of Interlaboratory Comparison Study Food VI (2013)

Detection of *Salmonella* spp. minced chicken meat

Day	Topic	Description
1	Pre-enrichment	Allow the BPW to equilibrate to at least room temperature Add 225 ml BPW to each plastic bag ('Whirl-pak') Mix (pulsifier or stomacher) sample Incubate at 37 °C ± 1 °C for 18 h ± 2 h
2	Selective enrichment	- 0.1 ml BPW culture in 10 ml RVS, incubate at 41.5 °C ± 1 °C for 24 h ± 3 h - 1 ml BPW culture in 10 ml MKTTn, incubate at 37 °C ± 1 °C for 24 h ± 3 h - 0.1 ml BPW culture on MSR/V plate, incubate at 41.5 °C ± 1 °C for 24 h ± 3 h
3	First isolation after 24 h	Inoculate from RVS, MKTTn, suspect MSR/V plates (24h): - XLD agar, incubate at 37 °C ± 1 °C for 24 h ± 3 h - Second isolation medium* (obligatory) * Incubate for specified time at the specified temperature
3	Continue selective enrichment	Incubate negative MSR/V plates another 24 h ± 3 h at 41.5 °C ± 1 °C
4	Second isolation after 48 h	If the first isolation was negative, inoculate from suspect MSR/V (48h) plates - XLD agar, incubate at 37 °C ± 1 °C for 24 h ± 3 h - Second isolation medium* * Incubate for specified time at the specified temperature
4	Confirmation of identity	Confirm the identity of the <i>Salmonella</i> suspect colonies from the first isolation media (day 3).
5	Confirmation of identity	Confirm the identity of the <i>Salmonella</i> suspect colonies from the second isolation media (day 4).

Annex A of SOP

Interlaboratory Comparison Study
Food VI (2013)Detection of *Salmonella* spp. in minced chicken meat