
PROTOCOL
INTERLABORATORY COMPARISON STUDY ON THE
DETECTION OF *SALMONELLA* spp. IN ANIMAL FEED
organised by EURL-*Salmonella*
ANIMAL FEED STUDY III - 2014

Introduction

This protocol describes the procedures for the third interlaboratory comparison study on the detection of *Salmonella* spp. in animal feed samples amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU.

The set-up of this study is comparable to the interlaboratory comparison studies on detection of *Salmonella* in food samples and samples from the primary production stage as organised in 2013 and 2014.

-The chicken feed samples are already spiked with *Salmonella* at the laboratory of the EURL-*Salmonella*. Hence the samples can be analysed 'as such' and no reference materials need to be added at the laboratory of the NRL.

-Less control samples are included and we ask each NRL to analyse the control sample(s) routinely used in the laboratory (e.g. a reference material or a (diluted) culture containing *Salmonella*).

The prescribed method is, like in earlier studies, the procedure as described in ISO 6579 ('Microbiology of food and feeding stuffs – Horizontal method for the detection of *Salmonella* spp.' Fourth edition, 2002). Additional to ISO 6579 it is requested also to use Annex D of ISO 6579 (EN-ISO 6579:2002/Amd1: 2007: Amendment 1: Annex D: 'Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage'). The method in this annex is especially intended for the detection of *Salmonella* spp. in animal faeces and environmental samples from the primary production stage, but it is also applicable for the analyses of animal feed samples. Additionally, laboratories (who are interested) can also perform an 'own' PCR method on the samples, if this is (routinely) used in their laboratories.

For the current study, it is no longer necessary to report results of 'own' culture media to the EURL-*Salmonella*. Results of an 'own' PCR method can still be recorded and will be used to compare the results with the results found with the bacteriological culture method.

The samples consist of 18 samples of chicken feed and 3 control samples. The samples are artificially contaminated with three different levels (blank, low and high level) of a *Salmonella* serovar (B1-B18). The control samples consist of one sample with only BPW (C1), one sample with chicken feed and BPW (C2) and one sample with an own positive control from the participating laboratory in BPW (C3). Each laboratory has to examine 21 samples in total.

Each box will be sent as biological substance category B (UN3373) by door-to-door (for non-EU-MS sometimes door-to-airport) courier service. Please contact EURL-*Salmonella* when the parcel has not arrived at your laboratory at 2nd of October 2014 (this is 3 working days after the day of mailing).

Objective

The main objective of the interlaboratory comparison study is to evaluate the performance of the NRLs for *Salmonella* for their ability to detect *Salmonella* spp. at different contamination levels in animal feed samples.

Outline of the study

Each participant will receive, in week 40 of 2014, one box containing 2 plastic safety bags, packed with cooling elements. The plastic safety bag contains:

21 numbered plastic 'Whirl-pak' bags:

- 18 plastic "Whirl-pak" bags numbered B1-B18;
- 3 plastic "Whirl-pak" bags numbered C1-C3;

Store the plastic bags at (5 ± 3) °C immediately after receipt.

One plastic safety bag will also contain the small electronic temperature recorder in a plastic bag with your lab code. Make a note of the lab code and **return the temperature recorder (in the plastic bag) to the EURL-*Salmonella* at the day your laboratory starts the study (6 October 2014)**. For this purpose the return envelope, with preprinted address label of the EURL-*Salmonella*, can be used.

The performance of the study will be in week 41 (starting on Monday 6 October 2014).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in animal feed III (2014) (this document);
- SOP Interlaboratory comparison study on the detection of *Salmonella* spp. in animal feed III (2014);
- Instructions for the web based test report Interlaboratory comparison study on the detection of *Salmonella* spp.: Guidance on submission of results EURL detection studies;

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- 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 faeces and in environmental samples from the primary production stage.
 - International Standard – ISO 6887-4: 2003 Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products and fish and fishery products.

The media to be used for this study will not be supplied by the EURL

This is the first EURL-*Salmonella* interlab comparison study for the detection of *Salmonella* in animal feed for which all data have to be reported through an electronic result form. The link, also to become available on the EURL-*Salmonella* website, and password for this form will be send by email to the participants. Submission of data has to be finalised on **27 October 2014 (23:59 h CET)** at the latest. **Mind that the electronic result form is no longer accessible after this deadline!** In case you foresee problems with the deadline, please contact us beforehand. The EURL will prepare a summary report soon after the study to inform all NRLs on the overall results.

Timetable of interlaboratory comparison study animal feed III (2014)Detection of *Salmonella* spp. in chicken feed

Week	Date	Topic
39	22 – 26 September	Mailing of the protocol, standard operating procedure (SOP) and instructions for the web based test report to the NRLs- <i>Salmonella</i> . Sending the link and the password for electronic reporting form to participants by email.
40	29 September	Mailing of the parcels to the NRLs as biological substance category B (UN3373) by door-to-door courier service. Immediately after arrival of the parcel at the laboratory: <ul style="list-style-type: none"> - Check for any serious damages and for completeness; (in case of deviations contact the EURL-<i>Salmonella</i>); - Store the samples at + 5°C ± 3 °C If you did not receive the parcel at 3 October, do contact the EURL immediately. Preparation of: <ol style="list-style-type: none"> 1. Non selective pre-enrichment medium (see SOP 6.1) 2. Selective enrichment media (see SOP 6.2) 3. Solid selective plating media (see SOP 6.3) 4. Confirmation media (see SOP 6.4)
41	6 October	Performance of the study, following the instructions as given in the protocol and the SOP of animal feed study III (2014). Remove the electronic temperature recorder from the safety bag (leave it in the plastic bag with lab code) and return it to EURL- <i>Salmonella</i> using the return envelope. Note the lab code before sending the recorder.
43	Deadline 27 October 23:59	Completion and submission of the web based test report. http://www.eurlsalmonella.eu/
	November 2014	Data elaboration at EURL- <i>Salmonella</i>
	December 2014	Sending of the individual laboratory results to the NRLs together with an interim summary report. As a follow-up, actions will be undertaken in case of poor performance.

If you have questions or remarks about this study, or in case of problems using the electronic reporting form, please contact:

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<http://www.eurlsalmonella.eu/>