



# *Salmonella* detection in spent irrigation water

## Introduction

In Regulation (EU) 209/2013, the microbiological criteria for sprouts are laid down, demanding the absence of *Salmonella* and Shiga toxin producing *E. coli* in five samples of each 25 g sprouts. This Regulation gives the food business operators producing sprouts the possibility to replace the sampling and testing of sprouts with the analysis of five samples of 200 ml of the water that has been used for their irrigation ('spent irrigation water'). For the detection of *Salmonella* reference is made to EN ISO 6579(-1), but no information is given on the pre-treatment of the irrigation water. Spent irrigation water may contain a high density of particle matter which may cause difficulties when using filtration for the concentration of *Salmonella* cells possibly present in the water. For that reason centrifugation may be a better method for concentrating the water sample. The EURL for *E. coli* has drafted a laboratory procedure for testing spent irrigation water for the presence of STEC, and their procedure for pre-treatment of the water samples by centrifugation is likely to be applicable for the detection of *Salmonella* as well. The procedure given below is based on the laboratory procedure of the EURL-VTEC (2020).

## Pre-treatment and analysis of spent irrigation water

Centrifuge the water samples in sterile tubes for at least 30 min at 4500 X g at 4 °C, preferably using a swing-out rotor in a refrigerated bench centrifuge. Decant carefully the supernatant. Suspend the total pellet in a sterile bottle or flask containing a quantity of pre-enrichment medium (BPW) to yield at least a tenfold dilution (or for example 100 ml). Incubate this initial suspension between 34 °C and 38 °C for 18 h ± 2 h.

For further analysis, follow the procedure as indicated in EN ISO 6579-1:2017/A1:2020 for food, animal feed samples, and environmental samples from the food production area (starting at 9.3.2).

Remark: It may be expected that the concentrated spent irrigation water samples contain high amounts of background flora. For that reason it is advisable to choose Modified semi-solid Rappaport-Vassiliadis (MSRV) agar as (first) selective enrichment medium additional to Muller Kauffmann tetrathionate-novobiocin (MKTTn) broth.

## References

Regulation (EU) No 209/2013 (2013) Amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and the sampling rules for poultry carcasses and fresh poultry meat. Official Journal of the European Union, L68, 12-03-2013. <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0209&rid=11> (access date 14-2-2022)

EN ISO 6579:2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.

EN ISO 6579-1:2017/A1:2020. Microbiology of food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. – Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC. International Organization for Standardization, Geneva.

EURL-VTEC, 2020. Laboratory procedure for testing spent irrigation water for the presence of STEC. EURL VTEC Method 09 Rev 1, 3/2/2020.

[https://www.iss.it/documents/5430402/0/EURL-VTEC\\_Method\\_09\\_Rev+1.pdf](https://www.iss.it/documents/5430402/0/EURL-VTEC_Method_09_Rev+1.pdf) (access date 14-02-2022).