Laboratory procedure for testing spent irrigation water for the presence of STEC

Introduction

Reg. (EU) 209/2013, laying down microbiological criteria for sprouts, 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. However, testing spent irrigation water for the presence of STEC or other enteric pathogens may pose technical problems, due to some characteristics of this particular matrix. In particular, the high density of the irrigation water, due to substances released by some species of sprouts, can make it difficult to use filtration for the concentration of the STEC bacterial cells possibly present in the water.

The present laboratory procedure represents an update of the methodology for pre-treatment of spent irrigation water samples to be entered in the analytical flow of the ISO/TS 13136:2012 standard previously issued by EURL-VTEC (EU-RL VTEC_Method_09_Rev 0, published on 23/01/2017).

The procedure comprises the following sequential steps:

- Centrifugation of water samples.
- Transfer of the resulting pellet presumptively containing STEC into the enrichment medium.
- Application of the ISO/TS 13136:2012 standard.

Procedure:

1. Pretreatment of water samples by centrifugation and enrichment of the resulting pellet

The water samples must be centrifuged for at least 30 min. at 4,500 X g at 4 °C. A swing-out rotor of a refrigerated bench centrifuge can be fit for the purpose. Pour the content of the bottle containing the test sample into suitable centrifuge tubes (*e.g.* 50 ml conical sterile disposable tubes) and centrifuge as indicated above. Carefully decant the supernatant and suspend the total pellet in a sterile bottle or flask containing an amount of enrichment medium corresponding to approximately 10 times the pellet volume or weight and incubate for 15-18 h at 41.5 °C. As far as the choice of the enrichment medium is concerned, we recommend the use of buffered peptone water (BPW).

2. Nucleic acid extraction, detection of virulence and serogroup-associated genes

These steps are performed according to the method ISO/TS 13136:2012, taking into account the EURL-VTEC adaptation for the detection of STEC O104:H4, available on the EURL website.

3. Isolation and identification of the pathogenic *E. coli* strains responsible for the positive PCR screening reactions

The enrichment cultures positive at the Real Time PCR screening step will be subjected to the isolation of the STEC strains responsible for the positive PCR results, according to the method ISO/TS 13136:2012.

Note: In case a serogroup-specific immunomagnetic separation is intended to be applied to aid the isolation of the STEC contaminating strain, take into account that highly dense water samples may generate highly dense enrichment cultures, and such a density might interfere with the proper functioning of magnetic beads. Therefore, in the case of a highly dense enrichment culture, it is advisable to perform a low speed centrifugation before proceeding with the serogroup-specific immuno-

magnetic separation. In this respect, a 5 ml aliquot of the enrichment culture is centrifuged at 500 X g for 1 minute, to sediment the dense particulate fraction. The immuno-magnetic separation is carried out on 1 ml of the resulting supernatant. This step aims at reducing the negative effects that the dense fraction could play in the adhesion of the beads to the magnet.

Evaluation of the performance

The present protocol has been applied in an inter-laboratory study, PT22, organized by the EURL-VTEC in 2018, in order to evaluate the performance of the procedure following the increase of the enrichment temperature at 41.5 °C. The report of the inter-laboratory study is available in the specific section of the EURL-VTEC website. The study consisted in the assessment of two different levels of contamination of sprout spent irrigation water samples with a STEC strain belonging to one of the serogroups included in the microbiologic criterion laid down by Reg. (EU) 209/2013, following the prescriptions of the same Regulation. Two irrigation water samples were sent to the laboratories that accepted to participate. One sample was uncontaminated, whereas the other was spiked with a STEC O103 strain at two different concentrations (0 CFU/ml and 50 CFU/ml). The results submitted by 50 laboratories, including NRLs of EU member States, NRLs of non-EU countries and Italian Official Laboratories were analysed and showed the following performance values.

Performance of the Real Time PCR detection of STEC-associated genes in the screening step in PT22

	Se	***Sp***
stx1	N.A.	99 %
stx2	84.7 %	100 %
eae	100 %	N.A.
wzx$_{O103}$	93.3 %	N.A.

During PT22, 63.4 % of the Laboratories succeeded in the isolation step, whereas in the previous PT on this matrix, PT19, which was based on the previous procedure, only 17 % of the participants could isolate the contaminating STEC strain, present in the same amount as in PT22 (50 CFU/ml).