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 concerns the pre-treatment methodology of spent irrigation water samples to be entered in the analytical flow of the ISO/TS 13136:2012 standard.

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.

The present protocol has been applied in an inter-laboratory study, PT16, organized by the EURL-VTEC in 2015, in order to evaluate the performance of the method. The report of the inter-laboratory study is available at the following link: http://www.iss.it/binary/vtec/cont/Report_PT16.pdf. A total of 51 laboratories, 30 NRLs of EU member States, 4 NRLs of non-EU countries and 17 Italian Official Laboratories (OLs) accepted to participate in PT16. The study consisted in the

assessment of 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. Three irrigation water samples were sent to the laboratories that accepted to participate. One sample was uncontaminated, whereas the remaining two were spiked with a STEC strain at two different concentrations (200 CFU/ml and 500 CFU/ml respectively). Results were collected by 50 laboratories and one was considered as outlier. The data submitted by the remaining 49 showed the following performance values:

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

	Se (High)	Se (low)	Sp	Ac (High)	Ac (Low)
stx1	97,9 %	97,9 %	100 %	98,97 %	98,97 %
stx2	100 %	97,9 %	100 %	100 %	98,97 %
eae	97,9 %	97,9 %	N.A.	N.A.	N.A.
rfbE$_{O157}$	97,9 %	97,9 %	N.A.	N.A.	N.A.

STEC O157 isolation step

- Se: 89.8 % (high level) and 85.7 % (low level).

Overall, the analytical results provided by the participating laboratories, confirmed the suitability of the the developed procedure for the pre-treatment of spent irrigation water samples.

Procedure:

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

Centrifuge the water samples in sterile tubes for at least 30 min at 4,500 X g at 4 °C, preferably using a swing-out rotor in a refrigerated bench centrifuge. Decant carefully

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/weight and incubate for 18-24 h at 37 °C ± 1 °C.

As far as the choice of the enrichment medium is concerned, we recommend to use buffered peptone water (BPW).

2. Nucleic acid extraction, detection of virulence and serogroup-associated genes and isolation and identification of the pathogenic *E. coli* strains responsible for the positive PCR screening reactions

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

Note: Spent irrigation water samples may generate highly dense enrichment cultures, and such a density might interfere with the proper functioning of magnetic beads, if a serogroup-specific immuno-magnetic separation procedure is applied to facilitate the isolation of the STEC strain. Therefore, in such a case 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.

As a back-up procedure, it is recommended to perform the direct plating of the enrichment culture in parallel to the IMS in order to screen the colonies for the *stx*-genes in case of a negative IMS.