Detection of Enteroinvasive *Escherichia coli* in food by Real Time PCR amplification of the *ipaH* gene

1. Aim and field of application

Enteroinvasive *Escherichia coli* (EIEC) represent a group of diarrheagenic *E. coli* biochemically, genetically, and pathogenetically related to *Shigella* spp. Both pathogens are characterised by the ability to invade the colonic epithelium, determined by the presence of genes located either on a large invasion plasmid, pINV, or on the bacterial chromosome.

EIEC infections are mainly reported in countries with poor sanitation and socio-economic status, while in industrialised countries they are usually sporadic and travel-related. However, food-borne and water-borne outbreaks have been reported in non-endemic countries, such as the United States, Japan Israel and Europe. Moreover, EIEC infections may be confused with shigellosis, and their prevalence may be underestimated. Therefore, the procedures allowing the detection of EIEC in foodstuffs should be available in public health laboratories.

The present procedure describes a molecular methodology to screen food samples for the presence of EIEC by the detection of a target designed on the invasion plasmid antigen H (*ipaH*) gene, which represent a genetic marker characteristic for this group of pathogenic *E. coli* as well as for *Shigella* spp. The same genetic marker is currently used for the detection of *Shigella* spp. and EIEC in the faeces of patients with diarrhea.

2. Screening of food samples

Enrichment cultures are obtained by adding 25 gr test portions of the food specimen (or 25 ml of liquid) to 225 ml of Buffered Peptone Water (BPW), homogenizing in a peristaltic blender, and incubating at 37 ±1 °C for 18-24 h.

One ml of the enrichment culture is used for DNA extraction. This step is accomplished by any method in use in the laboratory for the extraction and purification of DNA from food enrichment cultures.

The Real Time PCR targeting the *ipaH* gene is performed with the primers and probe reported in **Table 1**. Amplification conditions will depend on the system used and will refer to the instructions supplied with the instrument and the kit of choice, and should be set up in each laboratory. However, standard reaction conditions together with a two-steps thermal profile applied at EU-RL VTEC are provided.

Enrichment cultures positive for the presence of *ipaH* are streaked onto suitable solid media (MacConkey agar plates or other media suitable for *E. coli* isolation, such as TBX) for attempting the isolation. This step is accomplished as follows:

- ❑ Pick up to 50 colonies with *E. coli* morphology.
- ❑ Point-inoculate on Nutrient Agar (NA) (single colonies).
- ❑ Test the isolated colonies or pools of 10 colonies by real time PCR for the presence of the *ipaH* gene.
- ❑ Subculture the positive colony for further characterisation.

3. Real Time PCR amplification of the *ipaH* gene

The protocol is based on the 5'-nuclease PCR assay. The primers and probe targeting the *ipaH* gene, have been described by Wang et al. (2010) and their sequences are reported in the table below.

The nature of the Reporter and Quencher is not indicated, as it may depend on the Real Time PCR apparatus available in the laboratory.

An internal amplification control (IAC) must be run simultaneously, to identify possible inhibition of the test samples.

Table 1. DNA sequence and characteristics of the primers and probe used for the detection of EIEC.

Target gene	Primer/Probe name	Forward Primer, Reverse Primer and Probe sequences (5'-3')	Amplicon size (bp)	Location within sequence	Sequence Acc.No.
ipaH	*ipaH-U1* FWD	**CCT TTT CCG CGT TCC TTG A**	63	1065-1083	M32063
	ipaH-L1 REV	**CGG AAT CCG GAG GTA TTG C**		1128-1110	
	ipaH-P1 probe	**CGC CTT TCC GAT ACC GTC TCT GCA**		1085-1108	

The Real Time PCR conditions are as follows:

Master Mix 2X	to 1X (usually containing $MgCl_2$ to final concentration of 3mM)
Primer Fwd	500 nM
Primer Rev	500 nM
Probe	200nM
DNA	X (2 μl of DNA purified from 1 ml of culture can be sufficient)
Water	to final volume

The primers and probes have been evaluated at the EU-RL VTEC with a Corbett Rotorgene, by using the following basic two steps thermal profile:

95°C 10 minutes

35-40 cycles of:

- 95°C 15 seconds
- 60°C 60 seconds

4. References

Buysse JM, Stover CK, Oaks EV, Venkatesan M, Kopecko DJ. Molecular cloning of invasion plasmid antigen (*ipa*) genes from *Shigella flexneri*: analysis of *ipa* gene products and genetic mapping. J Bacteriol. 1987;169:2561-9.

Gordillo ME, Reeve GR, Pappas J, Mathewson JJ, DuPont HL, Murray BE. Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas. J Clin Microbiol. 1992; 30: 889-93.

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Wang SM, Ma JC, Hao ZY, Zhang ZY, Mason C, Sethabutr O, von Seidlein L, Wang XY, Xu ZY. 2010. Surveillance of shigellosis by real-time PCR suggests underestimation of shigellosis prevalence by culture-based methods in a population of rural China. J Infect. 61(6):471-475.