

Detection of Enteroaggregative *Escherichia coli* in food by Real Time PCR amplification of the *aggR* and *aaiC* genes

1. Aim and field of application

The large outbreak occurred in Germany during Summer 2011 associated to the consumption of sprouts was caused by a VTEC strain which possessed atypical characteristics, and did not fit the current definition of pathogenic VTEC. In fact, the epidemic strain did not possess the *eae* gene, encoding the adherence factor intimin, but possessed genes that are typical of the Enteroaggregative *Escherichia coli* (EAggEC) group. The strain was indeed an EAggEC that had acquired the *stx2a*-converting bacteriophage. This matter has highlighted the importance to screen foodstuffs for EAggEC too, which may represent an emerging public health concern.

The present procedure describes a molecular methodology to screen food samples for the presence of EAggEC by the detection of targets designed on the *aggR* and *aaiC* genes, which represent genetic markers characteristic for this group of pathogenic *E. coli*. The same genetic markers have been indicated by the European Centre for Disease Prevention and Control (ECDC) and by the European Food Safety Authority (EFSA) for the identification of EAggEC strains.

2. Screening of food samples

Enrichment cultures are obtained by adding 25 g 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 *aggR* and *aaiC* genes is performed with the primers and probes reported in Annex 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 included in Annex 1.

Enrichment cultures positive for the presence of *aggR*, or *aaiC*, or both genes 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 gene(s) detected in the screening step.
- Subculture the positive colony for further characterisation.

3. Real Time PCR amplification of the *aggR* and *aaiC* genes

The present annex illustrates the primers and probes sequences and the Real Time PCR conditions for the amplification of two genetic markers of typical EAggEC: the plasmid-located gene *aggR*, coding for a transcription regulator, and the chromosomal gene *aaiC*, which is part of the *aai* gene cluster, encoding a type VI secretion system.

The protocol is based on the 5'-nuclease PCR assay. The primers and probes targeting the *aggR* and *aaiC* genes have been deployed and tested at the EU-RL VTEC 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.

The amplification of the two target genes can be performed simultaneously in a triplex Real Time PCR, including the assays for the two genes and the internal amplification

control (IAC), if the Real Time Apparatus allows the simultaneous detection of three different fluorophores.

Table. DNA sequence and characteristics of the primers and probes used for the detection of EAggEC.

Target gene	Primer/Probe name	Forward Primer, Reverse Primer and Probe sequences (5'-3')	Ampli con size (bp)	Location within sequence	Sequence accession number
<i>aggR</i>	<i>aggR</i> FWD	GAATCGTCAGCATCAGCTACA	102	47738-47718	CU928159.2
	<i>aggR</i> REV	CCTAAAGGATGCCCTGATGA		47637-47656	
	<i>aggR</i> probe	CGGACAACCTGCAAGCATCTA		47697-47678	
<i>aaiC</i>	<i>aaiC</i> FWD	CATTTACAGCTTTTTTCAGGAAT	160	3385498-3385477	NC_011748.1
	<i>aaiC</i> REV	CCTGATTTAGTTGATTCCCTACG		3385339 - 3385361	
	<i>aaiC</i> probe	CACATACAAGACCTTCTGGAGAA		3385427-3385405	

Real Time PCR conditions:

Master Mix 2X to 1X (usually containing MgCl₂ 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
 - 58 °C 60 seconds

4. References

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