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A Modular approach in method validation of the real-time PCR for the detection of five VTEC Escherichia coli serogroups

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Introduction

The detection and identification of pathogenic microorganisms in food products often requires the simultaneous demonstration of distinct DNA sequences in a single viable organism. While the latter can only be demonstrated with classical microbiological assays, combined PCR analyses allow for a very sensitive detection of multiple pathogenic sequences in a sample. In the field of genetically modified organisms (GMO), minimal critical performance parameters have been established for PCR GMO detection methods. The PCR efficiency, method specificity and sensitivity are recognized as key parameters for qualitative PCR detection methods in GMOs. Method validation in GMO analysis applied successfully a so-called 'modular approach'. Here we demonstrate that such approach allows for *in-house* validation of an integrated set of real-time PCR methods proposed to CEN (CEN TC275/WG6) for the detection of Verotoxigenic *Escherichia coli (VTEC).* A proposal for a combinatory PCR decision support system allowing detection and identification of different VTEC serogroups is presented.

Methods

❖ Eight real-time TaqMan-based PCR modules were validated *in-house,* according to the criteria set by the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF) http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

❖ The targets included specific serogroups and toxin genes

❖Efficiency, dynamic range, specificity and Limit of detection (LOD) were evaluated. For the LOD 60 repetitions were done (Table1)

❖ Spiked milk samples were also analyzed to check the possible "matrix effect"

Results and discussion

❖ All modules performed well for qualitative purposes (efficiency rates E>90% apart from two methods) The modules were also tested for matrix effect (spiked and non-spiked milk samples) and no interference of the matrix was observed

❖ The modules were highly specific, no aspecific amplifications were observed

Table 1. Values of 60 repetitions to determine the LOD of stx1, stx2 and eae PCR modules. Arrows indicate the copy number at which the LOD was set

Module	Copy number per reaction	Average Ct±SD	Positive (%)
stx1	15	34±0.9	60/60 (100%)
LOD→	10	36.5±1.22	57/60 (95%)
	5	37.5±1.29	52/60 (87%)
	0.1	42.3±1.47	3/60 (5%)
stx2	15	34.1±0.78	60/60 (100%)
LOD→	10	36.4±1.34	59/60 (98%)
	5	38.3±1.61	56/60 (93%)
	0.1	41.4±1.07	7/60 (12%)
eae	15	34.8±1.49	60/60 (100%)
	10	35.9±1.04	60/60 (100%)
LOD→	5	38.0±0.85	59/60 (98%)
	0.1	41.1±1.94	5/60 (8%)

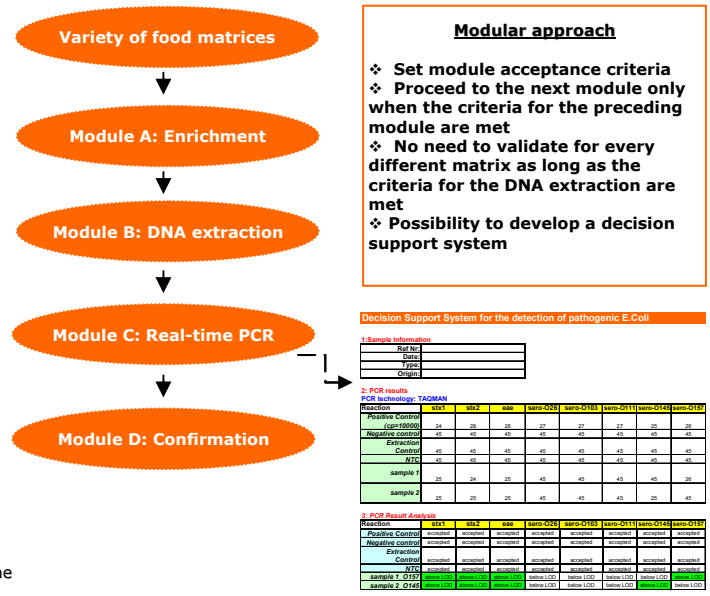

Figure 1. Example of the modular approach in food microbiology

Conclusion:
The concept of modularity could be successfully applied to the validation of PCR-based food microbiology methods resulting in a more cost effective validation approach

Future perspectives:
Optimization of the combinatory feasibility of the PCR methods into a single 96-well format and development of low-cost tools for routine analysis of food samples for VTEC presence (ready-to-use PCR plates)
High resolution distinction of all types of VTEC in samples

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