Validation of GENE UP® EHEC Method for STEC Detection Using New Molecular Makers and Isolation on IDEA™ STEC Agar

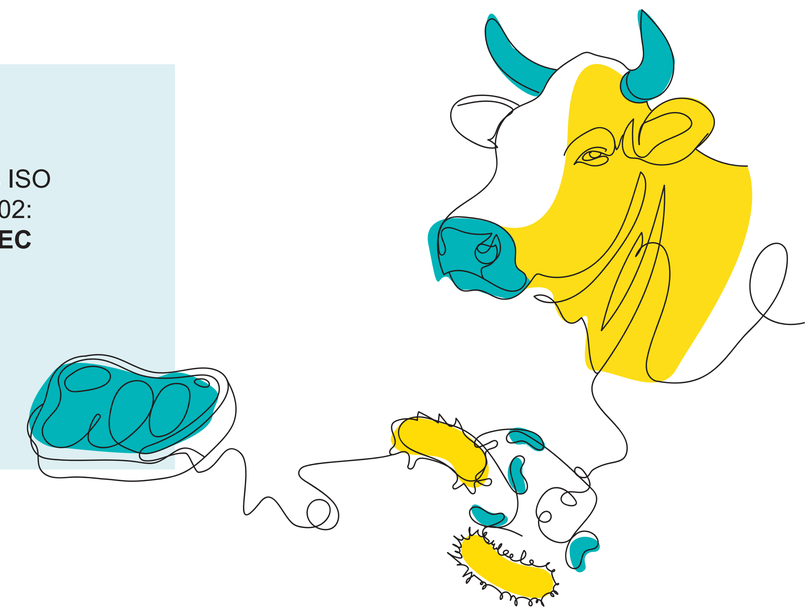
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INTRODUCTION AND OBJECTIVE

EHEC are major foodborne pathogens requiring rapid and accurate detection. Novel Molecular markers *(espK / espV)* are suggested to improve the specificity of EHEC by detecting colocalization of *stx* and *eae* genes in the same cell [1] [2].

IDEA™ STEC Agar is designed to enable faster, clearer, and more reliable isolation of STEC and particularly EHEC strains based on detection of Shiga toxin production. New workflow combines GENE-UP® Pathogenic *E. coli* kit (PEC) + IDEA™ STEC Agar in the previous validated method including *stx* and *eae* and TOP 7 serogroup detection.

> Objective: validate new workflow vs ISO 131136: 2012 following ISO 16140-02: 2016 *E. coli* kit **(PEC) + IDEA™ STEC Agar.**



STUDY DESIGN

Variety of raw meat food sample inoculated with EHEC strains. New GENE-UP® EHEC workflow compared to ISO/TS 13136: 2012 [4]
Interpretated with vs without PEC kit
Ten additional samples were co-inoculated with two strains, one *stx* only and one *eae* only, to evaluate PEC performance in reducing false positives. IDEA™ STEC Agar tested in parallel to other chromogenic agars.

Sensitivity

60 samples tested
- 36 — Inoculated at low level (**<3CFU/ test portion**)
- 24 — Not inoculated

RLOD

30 samples /Matrix
- 5 — Not inoculated
- 20 — Inoculated at low level with *E. coli O103* (**0.7 CFU/test portion**)
- 5 — Inoculated at high level with *E. coli O103* (**2.9 CFU/test portion**)

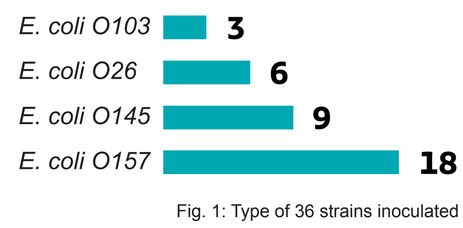


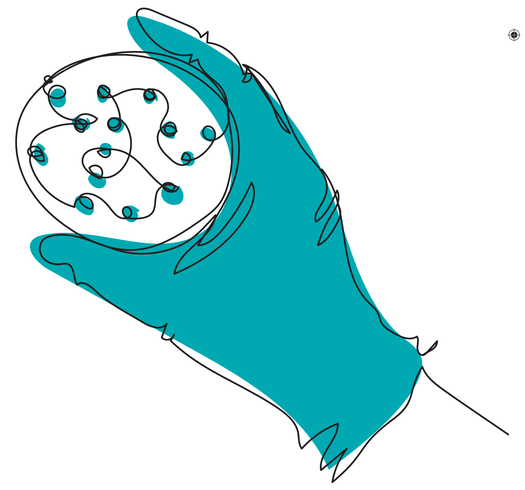
Fig. 1: Type of 36 strains inoculated

RLOD STUDY RESULTS

RLOD values meet acceptability criteria (≤2.5).

(Strain/Matrix) pair	RLOD (95% confidence limit)					Level of detection at 50% (CFU/test portion)	
	AL	Without PEC		With PEC		Reference method	Alternative method (at 8 h and 24 h, with or without PEC)
		8 h	24 h	8 h	24 h		
Ground beef 15% fat/ *E. coli O103* (stx1/stx2/eae) Ad3258	2.5	0.3 (0.1;0.3)	0.3 (0.1;0.6)	0.3 (0.1;0.6)	0.3 (0.1;0.6)	1.7 (0.9;3.6)	0.4 (0.2;0.7)

Table 1: RLOD and LOD50 results for evaluated category



SENSITIVITY STUDY RESULTS

16 discordants were obtained, with 11 positive deviations in favor of the alternative method. Comparable performance with/without PEC, not producing more negative deviations

Among the 10 co-inoculated samples tested, false-positive results were observed in 6 samples without PEC and 5 samples with PEC. PCR-positive samples confirmed on IDEA™ STEC: 27/28 (8 h); 28/29 (24 h).

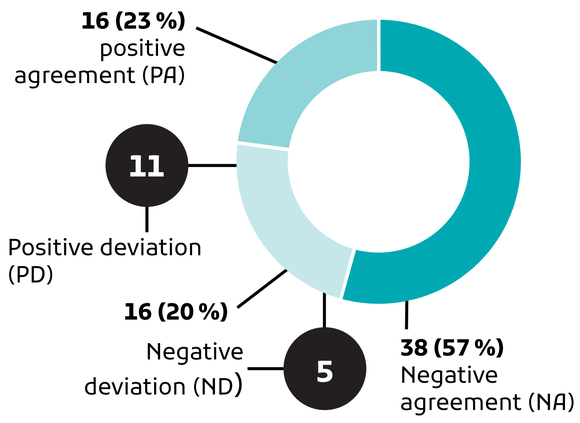


Fig. 1: repartition of the results of the sensitivity study



Fig. 2: IDEA STEC plate: typical colonies surrounded by halos.

	Category	Enrichment protocol	total N+	TND-PD	SE_{alt} %	SE_{ref} %	RT %	FPR %
Without PEC	Raw meat (up to 25 g)	Pre-warmed BPW 8 h at 41,5 °C	32	-6	84.4	65.6	73.3	3.6
		Pre-warmed BPW 24 h at 41,5 °C	32	-7	84.8	63.6	71.7	3.7
With PEC	Raw meat (up to 25z)	Pre-warmed BPW 8 h at 41,5 °C	32	-6	84.4	65.6	73.3	3.6
		Pre-warmed BPW 24 h at 41,5 °C	33	-7	84.8	63.6	71.7	3.7

SEalt = sensitivity for the alternative method - SEref = sensitivity fot the reference method
RT = Relative Trueness - FPR = faise positive ratio for the alternative method

Table 2: Data analysis for tested category

INCLUSIVITY/EXCLUSIVITY EVALUATION

Inclusivity: 48/50 strains showed the expected positive results across all PCR kits (including PEC) and confirmation steps (typical colonies on IDEA STEC).

Exclusivity: 34/34 strains gave the expected negative results according to the full workflow.

CONCLUSION

New workflow shows equivalent performance to ISO 13136: 2012. Alternative method is selective and specific with or without the use of PEC and IDEA STEC. Suitable for routine STEC detection.

High sensitivity & specificity. PEC performance should be further evaluated using naturally contaminated samples.

References

[1] Bosilevac, J.M.; Katz, T.S.; Manis, L.E.; Rozier, L.; Day, M. Using Pathogenic Escherichia coli Type III Secreted Effectors *espK* and *espV* as Markers to Reduce the Risk of Potentially Enterohemorrhagic Shiga Toxin-Producing *Escherichia coli* in Beef. Foods 2025, 14, 382.

[2] Delannoy, S.; Chaves, B.D.; Ison, S.A.; Webb, H.E.; Beutin, L.; Delaval, J.; Billet, I.; Fach, P. Revisiting the STEC Testing Approach: using *espK* and *espV* to Make Enterohemorrhagic *Escherichia coli* (EHEC) Detection More Reliable in Beef. Front. Microbiol. 2016, 7.

[3] ISO 16140-2: 2016; Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

[4] ISO 13136: 2012; Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of *O157*, *O111*, *O26*, *O103* and *O145* serogroups.

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