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Instituut voor Landbouw- en Visserijonderzoek

# Thorough identification of pathogenic Shigatoxin producing *Escherichia coli* (STEC) in Belgium

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**10th Annual workshop of the NRLs for *E. coli* in the EU**
**5-6 November 2015**

IDESTEC 2 RT 12/12
2010-2014

.be

# Regulation

- Regulation EC No. 209/2013 as regards microbiological criteria for sprouts (1/07/2013): Absence of STEC O157, O26, O111, O103, O145 and O104:H4 in 25g

the following row 1.29 and the corresponding footnotes 22 and 23 are added:

| | | | | | | |
|---|---|---|---|---|---|---|
| '1.29 Sprouts ([23]) | Shiga toxin producing *E. coli* (STEC) O157, O26, O111, O103, O145 and O104:H4 | 5 | 0 | Absence in 25 grams | CEN/ISO TS 13136 ([22]) | Products placed on the market during their shelf-life |

([22]) Taking into account the most recent adaptation by the European Union reference laboratory for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC), for the detection of STEC O104:H4.

([23]) Excluding sprouts that have received a treatment effective to eliminate *Salmonella* spp. and STEC.';

11.03.2013

# IDESTEC 2 12/12

Enrichment

↓

Extraction gDNA

↓

qPCR Screening

↓ if screening positive

Isolation

↓

Confirmation

↓

Further characterisation

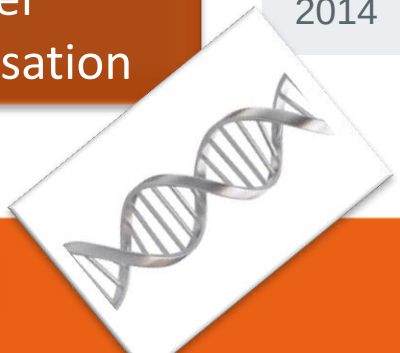
TECHNICAL SPECIFICATION **ISO/TS 13136**

First edition
2012-11-15

**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**

*Microbiologie des aliments — Méthode basée sur la réaction de polymérisation en chaîne (PCR) en temps réel pour la détection des micro-organismes pathogènes dans les aliments — Méthode horizontale pour la détection des Escherichia coli producteurs de Shigatoxines (STEC) et la détermination des sérogroupes O157, O111, O26, O103 et O145*

| year | # samples tested | Presumptive Detection VTEC | VTEC Detected |
|---|---|---|---|
| 2013 | 1722 | 162 (9.4%) | 16 (0.9%) |
| 2014 | 1793 | 229 (12.7%) | 31 (1.7%) |



# Comparison of methods

4 non-O157 STEC strains (O26, O103, O111, O145) ± 10 cfu/25g

**Artificial** contaminated food matrices

**Detection**

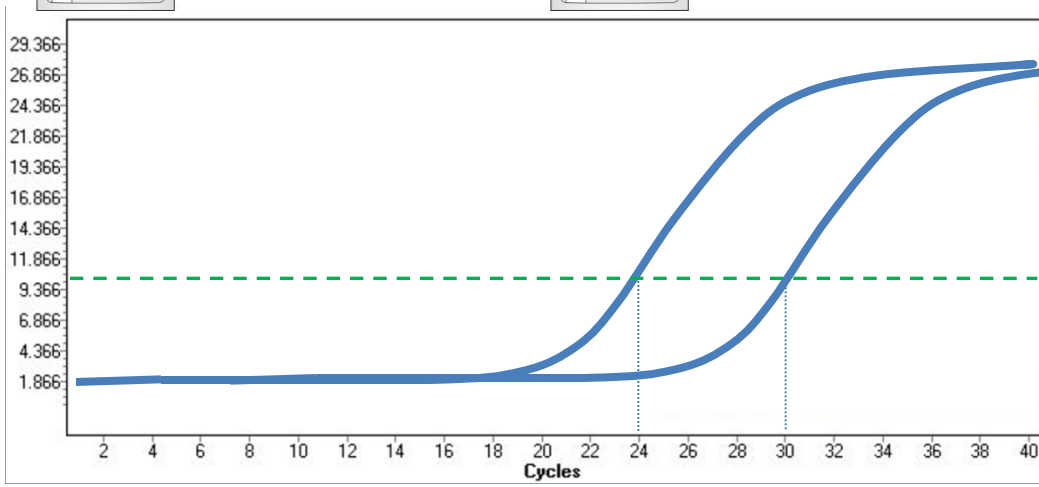
BPW

NucleoSpin Food kit

Real-time qPCR: (Verstraete et al., 2014)

Efficient enrichment

Less efficient enrichment

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# Comparison of methods

4 non-O157 STEC strains (O26, O103, O111, O145) ± 10 cfu/25g

**Artificial** inoculated food matrices

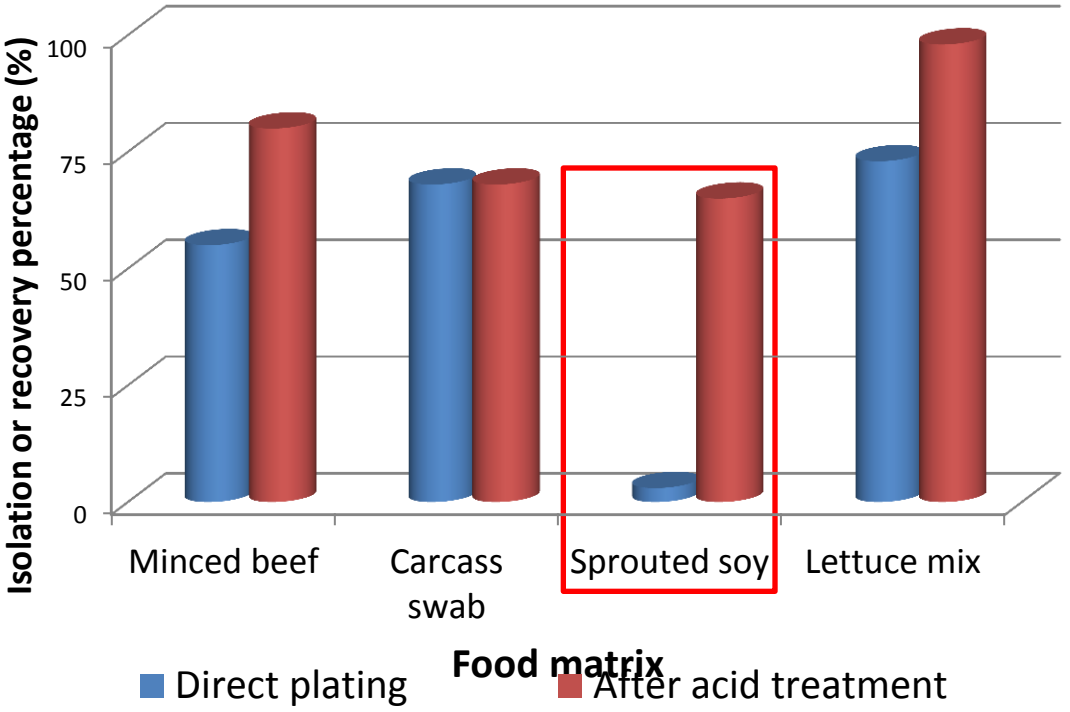
**Isolation**

BPW

Acid tr. (pH=2)

CHROMagar STEC and ChromID EHEC

Confirmation

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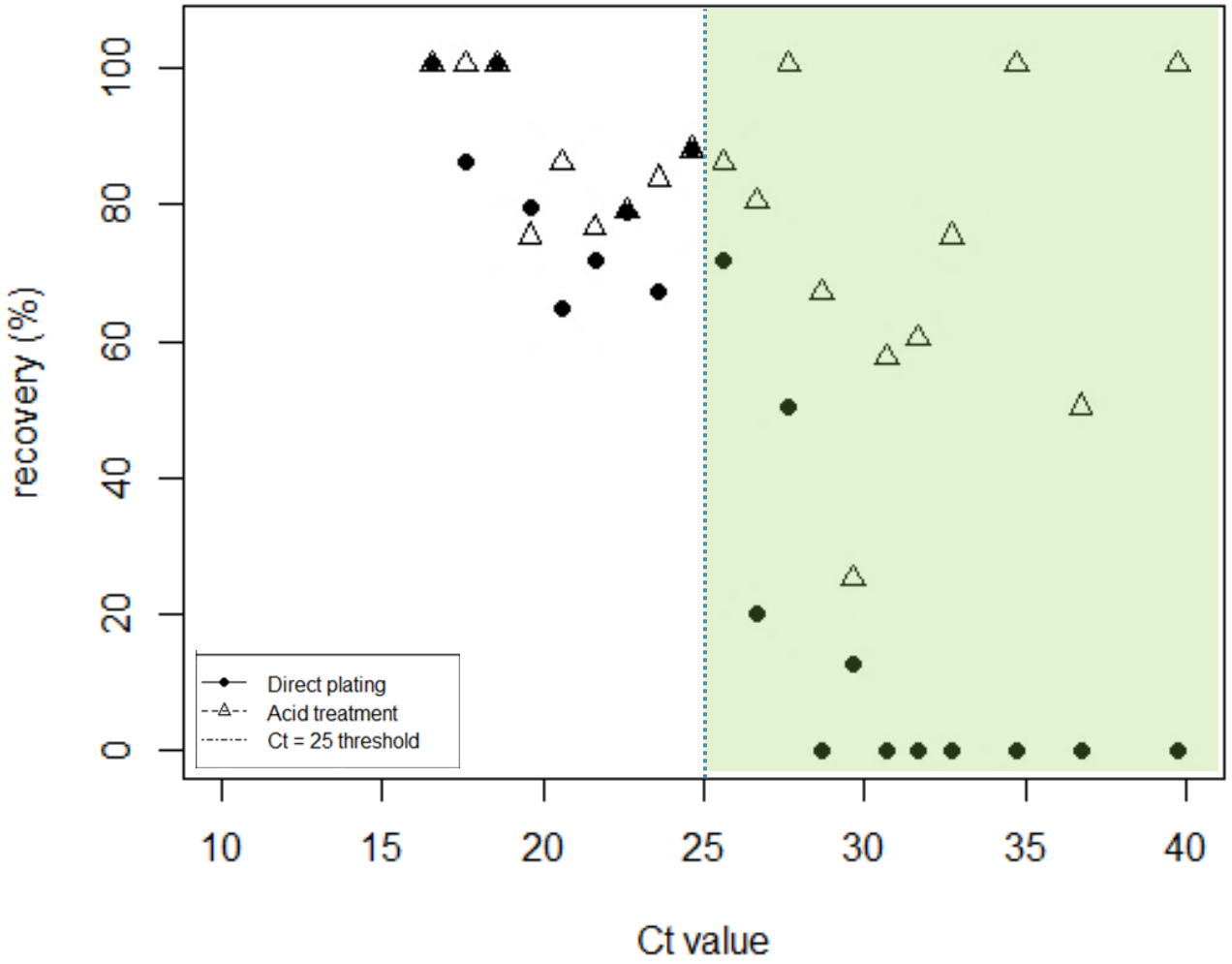


## Ct > 25:

- = Less efficient enrichment
- More interfering background microbiota

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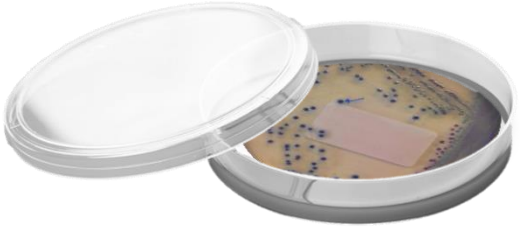
High selective agar medium

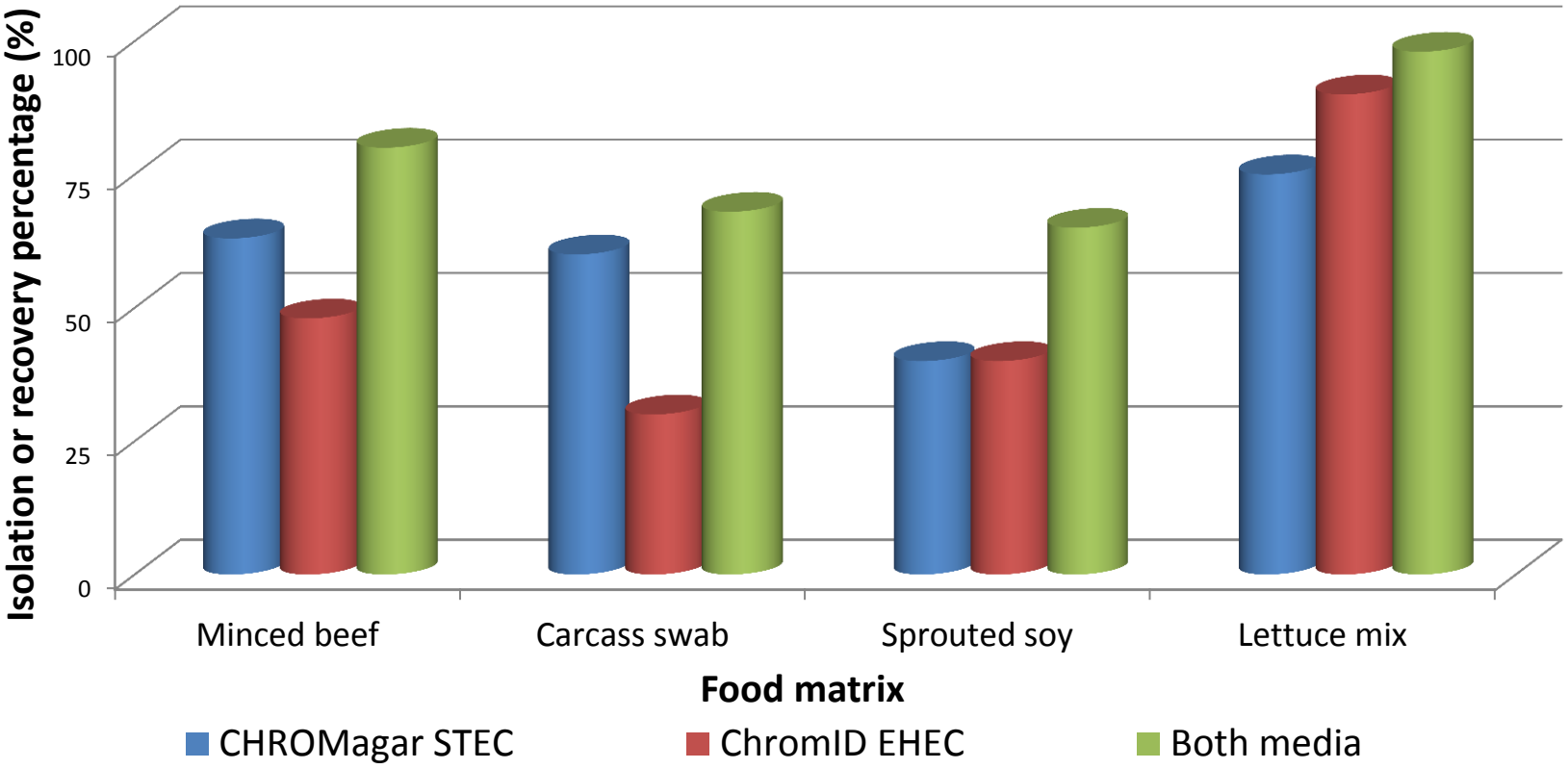
(CHROMagar STEC)

+



Less selective agar medium

(ChromID EHEC)




Combination of both isolation media

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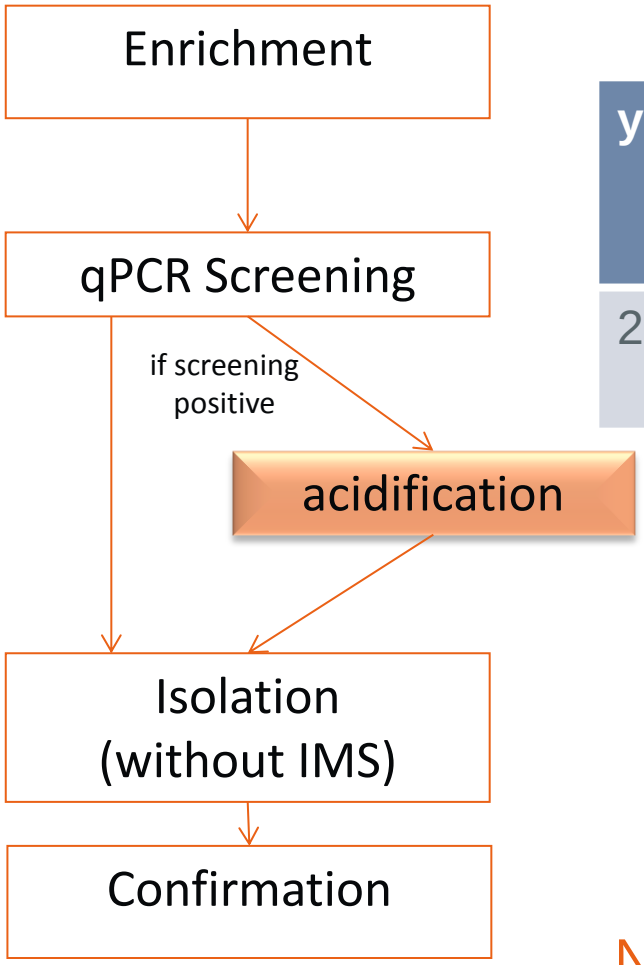
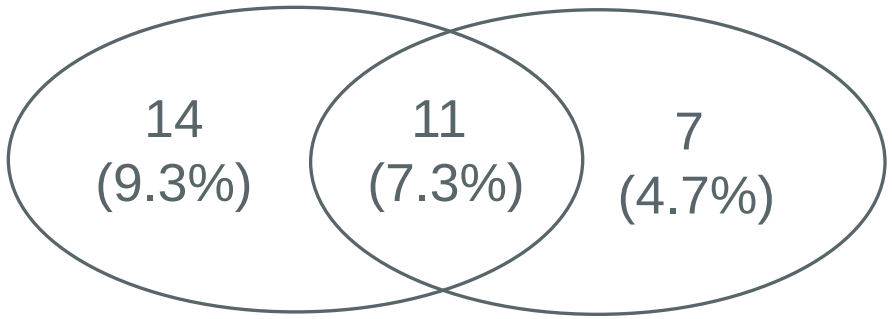
# Naturally contaminated samples




## qPCR screening and isolation

| year | Samples tested | Presumptive detection VTEC | STEC Detected |
|---|---|---|---|
| 2014 | 1793 | 229 (12.7%) | 31 (1.7%) |

## pH- treatment (N=150 samples)



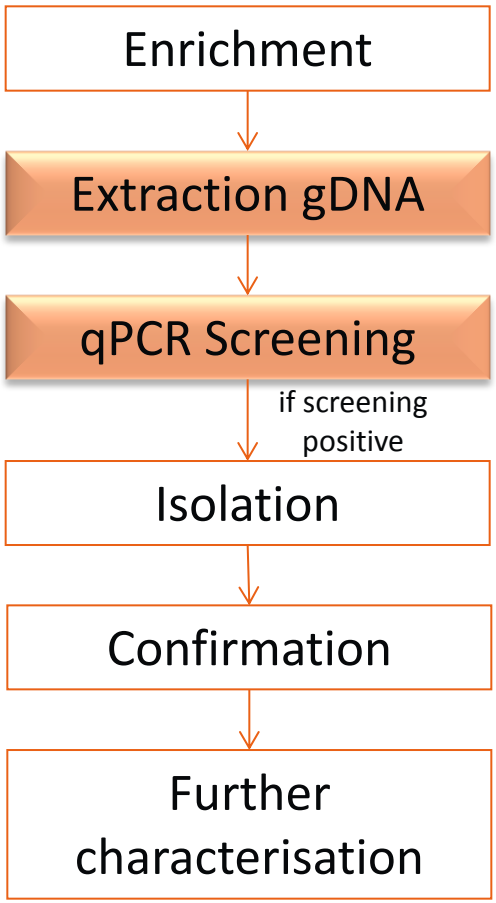

No treatment

Acid treatment



Verhaege et al. Submitted for publication

# II. qPCR screening method for VTEC




- Comparison 2 gDNA extraction methods

| | Quality | Quantity | Price | Time |
|---|---|---|---|---|
| FEP | + | x1 | 1.7€/sample | 10min |
| NSFK | +++ | x30 | 2.52€/sample | 3h |

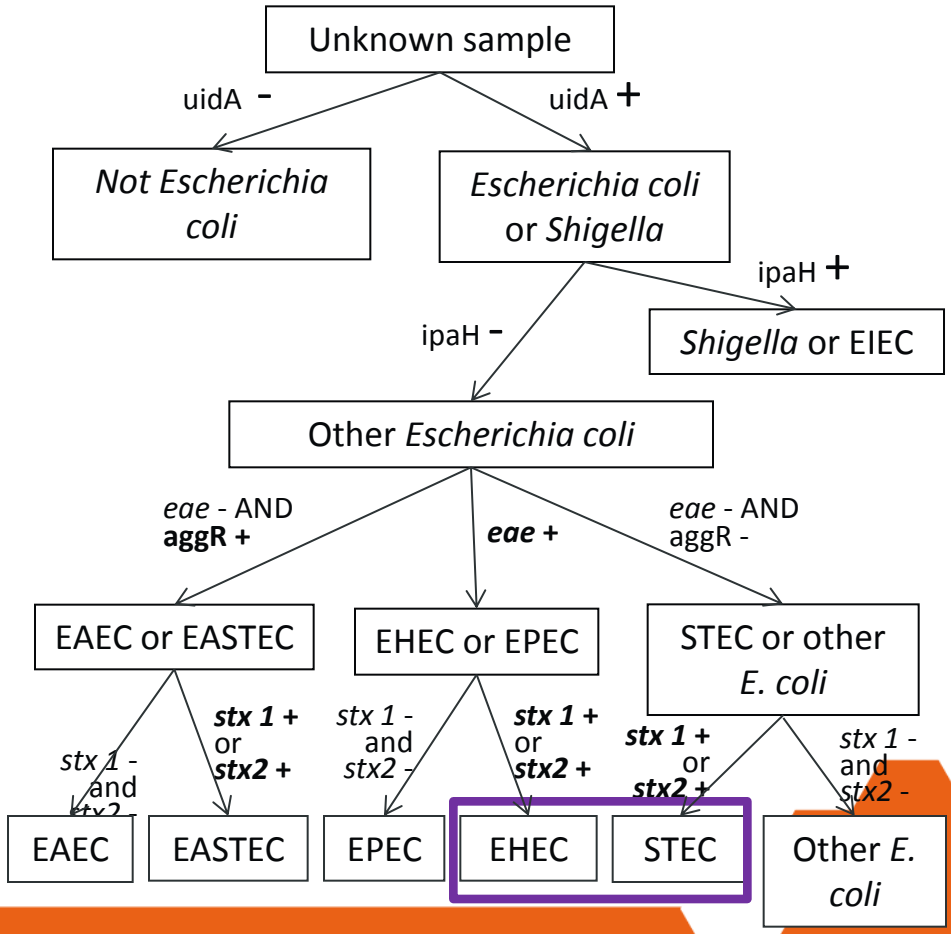
- qPCR Screening targeting *stx1, stx2* and *eae* genes
  - ✓ CoSYPS Path *E. coli* (WIV-ISP)
  - ✓ GeneDisc STEC (Pall®)
  - ✓ qPCR Verstraete *et al*, 2012 (ILVO)
  - ✓ Foodproof STEC Screening (BIOTECON Diagnotics)

# Comparison different qPCR Screening systems

| | | | GeneDisc® STEC | Verstraete et al 2012 | CoSYPS Path *E. coli* | Foodproof® STEC Screening LyoKit |
|---|---|---|---|---|---|---|
| Designer | | | Pall | ILVO | WIV-ISP | Biotecon |
| Real-Time PCR platform | | | GeneDisc® | All | All | All |
| *E. coli* | *uidA* | | | | X | |
| STEC | *stx1* | | X | X | X | X |
| | *stx2* | *stx2 a,b,c,d,e,g* | X | X | X | X |
| | | *stx2f* | | | X | X |
| EHEC | *eae* | | X | X | X | X |
| | *ehx* | | X | | | |
| EAggEC | *aggR* | | | | X | |
| EIEC & *Shigella* | *ipaH* | | | | X | |
| serogroups | O157 | | X | | | |

Piednoir et al
Submitted for publication

# qPCR Screening: CoSYPS Path *E. coli*



# III. Molecular characterisation of VTEC

## Strain collection: 757 strains- 773 gDNA

- Food (268), human (395) and animal (55) origins+ (45 unknown origin)
- 37 O-groups, 22 H-types | 5 pathotypes |19 virulence factors

## Genetical relation between STEC isolates in Belgium?

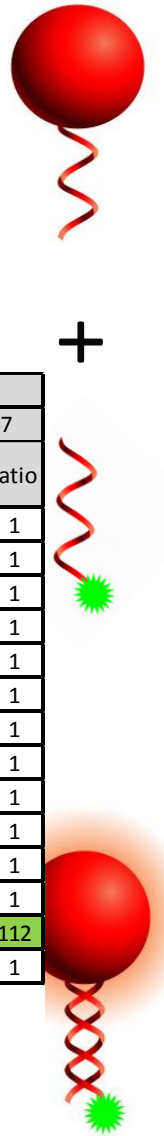
- PFGE, LSPA-6, Tir(A255T) SNP , Q21/Q933, Clade typing, *stx*2 subtyping

## Virulence profile of strains circulating in Belgium?

- Luminex

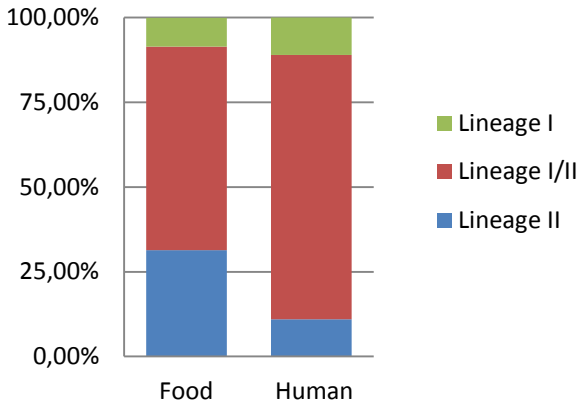
# List of the 42 genes in the VTEC characterization Luminex system.

| | Results of the 13 targets of the serogroup-13 assay | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | O26 | | O45 | | O55 | | O91 | | O103 | | O104 | | O111 | | O113 | | O118 | | O121 | | O128 | | O145 | | O157 | |
| Strain serogroup | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio | MFI | ratio |
| O26 | 2457 | 34 | 114 | 1 | 59 | 1 | 139 | 1 | 85 | 1 | 200 | 1 | 72 | 1 | 82 | 0 | 63 | 1 | 105 | 1 | 70 | 1 | 69 | 1 | 58 | 1 |
| O45 | 69 | 1 | 1206 | 11 | 69 | 1 | 142 | 1 | 83 | 1 | 183 | 1 | 68 | 1 | 83 | 0 | 56 | 1 | 102 | 1 | 81 | 1 | 78 | 1 | 63 | 1 |
| O55 | 66 | 1 | 110 | 1 | 3763 | 50 | 134 | 1 | 79 | 1 | 172 | 1 | 67 | 1 | 173 | 0 | 55 | 1 | 78 | 1 | 69 | 1 | 69 | 1 | 59 | 1 |
| O91 | 73 | 1 | 112 | 1 | 64 | 1 | 2840 | 25 | 89 | 1 | 202 | 1 | 80 | 1 | 100 | 0 | 64 | 1 | 103 | 1 | 83 | 1 | 83 | 1 | 71 | 1 |
| O103 | 65 | 1 | 92 | 1 | 62 | 1 | 112 | 1 | 1832 | 19 | 243 | 2 | 67 | 1 | 210 | 0 | 58 | 1 | 178 | 2 | 71 | 1 | 71 | 1 | 59 | 1 |
| O104 | 59 | 1 | 100 | 1 | 55 | 1 | 119 | 1 | 71 | 1 | 3029 | 19 | 63 | 1 | 75 | 0 | 55 | 1 | 84 | 1 | 63 | 1 | 67 | 1 | 53 | 1 |
| O111 | 72 | 1 | 107 | 1 | 67 | 1 | 129 | 1 | 79 | 1 | 173 | 1 | 1256 | 16 | 177 | 0 | 64 | 1 | 83 | 1 | 72 | 1 | 80 | 1 | 64 | 1 |
| O113 | 72 | 1 | 100 | 1 | 67 | 1 | 114 | 1 | 81 | 1 | 164 | 1 | 75 | 1 | 3035 | 6 | 71 | 1 | 92 | 1 | 79 | 1 | 81 | 1 | 69 | 1 |
| O118 | 62 | 1 | 100 | 1 | 64 | 1 | 119 | 1 | 71 | 1 | 242 | 2 | 67 | 1 | 81 | 0 | 7495 | 117 | 83 | 1 | 72 | 1 | 67 | 1 | 70 | 1 |
| O121 | 70 | 1 | 106 | 1 | 67 | 1 | 106 | 1 | 84 | 1 | 147 | 1 | 70 | 1 | 78 | 0 | 61 | 1 | 503 | 6 | 73 | 1 | 74 | 1 | 62 | 1 |
| O128 | 74 | 1 | 98 | 1 | 75 | 1 | 128 | 1 | 86 | 1 | 178 | 1 | 71 | 1 | 95 | 0 | 71 | 1 | 110 | 1 | 2883 | 35 | 75 | 1 | 63 | 1 |
| O145 | 71 | 1 | 95 | 1 | 62 | 1 | 105 | 1 | 76 | 1 | 150 | 1 | 69 | 1 | 276 | 1 | 65 | 1 | 100 | 1 | 85 | 1 | 1130 | 14 | 61 | 1 |
| O157 | 71 | 1 | 101 | 1 | 72 | 1 | 121 | 1 | 95 | 1 | 171 | 1 | 80 | 1 | 204 | 0 | 79 | 1 | 99 | 1 | 87 | 1 | 91 | 1 | 7786 | 112 |
| Blank | 73 | 1 | 107 | 1 | 75 | 1 | 115 | 1 | 98 | 1 | 161 | 1 | 77 | 1 | 497 | 1 | 64 | 1 | 88 | 1 | 82 | 1 | 82 | 1 | 70 | 1 |

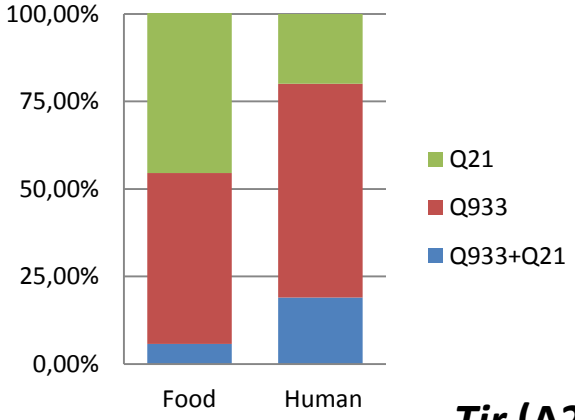


# Genetic diversity O157 STEC

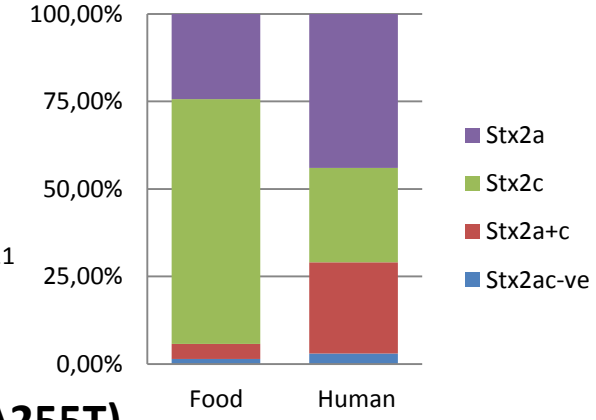
## LSPA-6



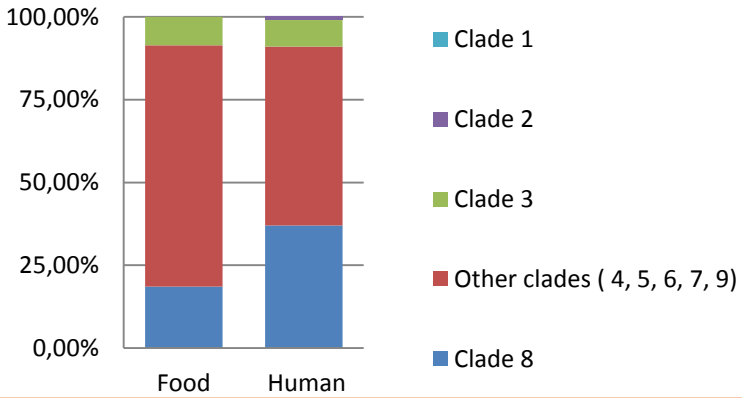

## Q21/Q933 alleles



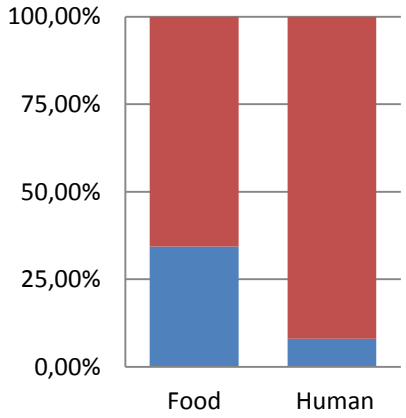

## *Stx* subtype




## Clade




## *Tir* (A255T)




Food, *n*=70
Human, *n*=100

# Conclusions

- Isolation method STEC on different matrices optimized
  - ⇒ pH treatment usefull to reduce high background flora
  - ⇒ Use of 2 isolation media is recommended
  - ⇒ IMS not specific enough to reduce background flora
- 4 qPCR screening methods for STEC validated and evaluated
- **Usefull for ISO/TS 13136 revision!**

- Typed strain collection (human, food, environment) available
- Luminex detection system for 42 genes developed

  Used for:
  - ⇒ Rapid detection of new emerging pathogens
  - ⇒ Outbreak investigation, trace back/forward
  - ⇒ Determination of reservoirs, transmission routes, virulence factors of STEC circulating in Belgium

*IDESTEC 2 12/12 was a collaboration of:*

Prof Dr. Lieven De Zutter (Ugent)

Dr. Marc Heyndrickx (ILVO)

Dr Ir Koen De Reu (ILVO)

**Bavo Verhaegen (Ugent/ILVO)**

Dr. Ir. Sigrid De Keersmaecker (WIV-ISP, PBB)

Dr. Katelijne Dierick (WIV-ISP, FoodPathogens)

**Dr. Elodie Barbau Piednoir (WIV-ISP)**

Dr. Nadine Botteldoorn (WIV-ISP)

Dr. Ir. Sarah Denayer (WIV-ISP, NRL VTEC)

*Acknowledgements:*

Dr K Verstraete (ILVO)

Dr M Elhadidy (Ugent/ILVO)

E Dumoleyn (ILVO)

E. Wattijn, W. Boukhouchi, E. De Vits, Z. Boukhouchi, C. Delsaut (WIV, VPA)

D. Van Geel (WIV, PBB)

NRC pathogenic *E. coli*

- Dr. Denis Pierard
- Klara De Rauw

NRL pathogenic *E. coli* France

- Dr Estelle Loukiadis

FASFC

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