

Detection of STEC: Alternative methods for industrial needs

October 6, 2023 | David Tomas. Scientific affairs

AGENDA

- INDUSTRIAL NEEDS
- METHOD VALIDATION
- ALTERNATIVE METHODS
- PERFORMANCE IMPROVEMENT

INDUSTRIAL FACTS

FOOD WASTE / FOOD SAFETY



HIGH VOLUME PRODUCTION

**SHORT SHELF LIFE /
DECISION TIME**

INDUSTRIAL ANALYTICAL NEEDS

RECOGNIZED / ACCURATE



EASY TO USE



FAST RESULTS



METHOD VALIDATION



REFERENCE AND ALTERNATIVE METHODS

TECHNICAL
SPECIFICATION

ISO/TS
13136

First edition
2012-11-15

COMMISSION REGULATION (EC) No 2073/2005
of 15 November 2005
on microbiological criteria for foodstuffs

Proprietary methods may be used as alternative analytical methods, provided they are:

- validated, in accordance with the protocol set out in standard EN ISO 16140-2, against the specific reference method provided for verifying compliance with the microbiological criteria laid down in Annex I, as provided for in the third subparagraph, and
- certified by an independent certification body.

**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**

VALIDATED ALTERNATIVE METHODS



CERTIFICATE OF COMPLIANCE

LLOYD'S REGISTER NEDERLAND B.V.

hereby declares that the certification assessment has demonstrated that

GENE-UP® EHEC Detection method

to be used with software versions 3.0 and 3.1

Manufactured by:
bioMérieux SA
Zone Polytec
5, rue des Berges
38000 Grenoble
France

Supplied by:
bioMérieux SA
376 Chemin de l'Orme
69280 Marcy L'Etoile
France

has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the MicroVal website:

www.microval.org

Reference method: ISO/TS 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

Scope: **raw meat**, except poultry (25 g and 375 g), **raw milk and raw milk cheeses and production environmental samples**

The validation and certification has been performed in accordance with ISO 16140-1:2016, **ISO 16140-2:2016** and the MicroVal Rules and Certification Scheme version 8.

VALIDATION ALTERNATIVE METHODS

EXPERT LABORATORY

- **SENSITIVITY STUDY**
 - 5 Food categories up to 18.
 - 3 Food types per category
 - TOTAL: 300 samples
- **RELATIVE LEVEL OF DETECTION (RLOD₅₀)**
 - 5 food categories up to 18.
 - 1 Food type per category
 - 3 spiking levels
 - TOTAL: 150 samples
- **INCLUSIVITY/EXCLUSIVITY**
 - 50 target strains
 - 30 non-target strains

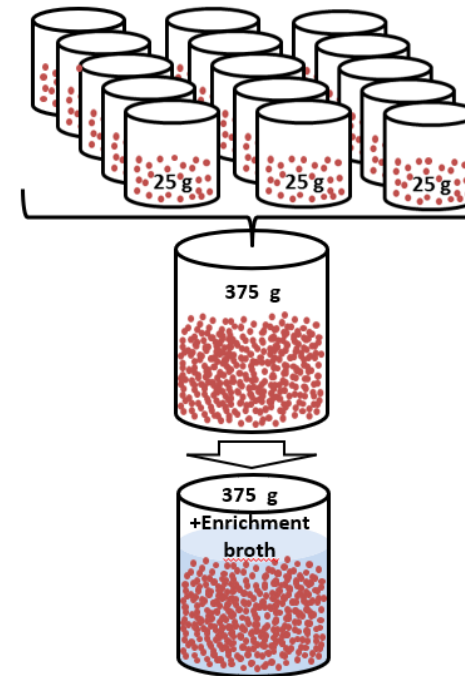
INTERLABORATORY

- **SENSITIVITY**
 - 1 Food item
 - 10 participant laboratories
 - 3 spiking level
 - TOTAL: 240 samples

ALTERNATIVE METHODS

VALIDATED ALTERNATIVE METHODS

- Five ISO 16140-2 validated alternative methods available in Europe for STEC detection.
- Including different scopes:
 - Raw beef (25 g and 375 g)
 - Raw milk and products from raw milk (25 g)
 - Vegetables (25 g and 375 g)
 - Environmental samples
 - Flour (375 g)
- Time to results from 8 hours (vs 18 hours).



SIMPLIFIED PROTOCOLS



Mix sample. Pipet enriched sample from filter side of bag



Transfer **10 μ L** to lysis tube using filtered tip



5 min @ 2200 rpm mechanical lysis

Easy sample preparation protocols

Tests list

Assay	Sample 2	Result	Interpretation	Stat
EH1	4	+	Presumptive presence of	

Tests detail

Name	Target r	Well	λ (nm)	CP	TM ($^{\circ}$ C)
sbx1/sbx2	+	D1	640	27.3	65.81
eee	-	D1	705	33.57	
IC	✓	D1	705	33.57	61.73

Tests list

Assay	Sample 2	Result	Interpre
EH1	12	-	Absence

Tests detail

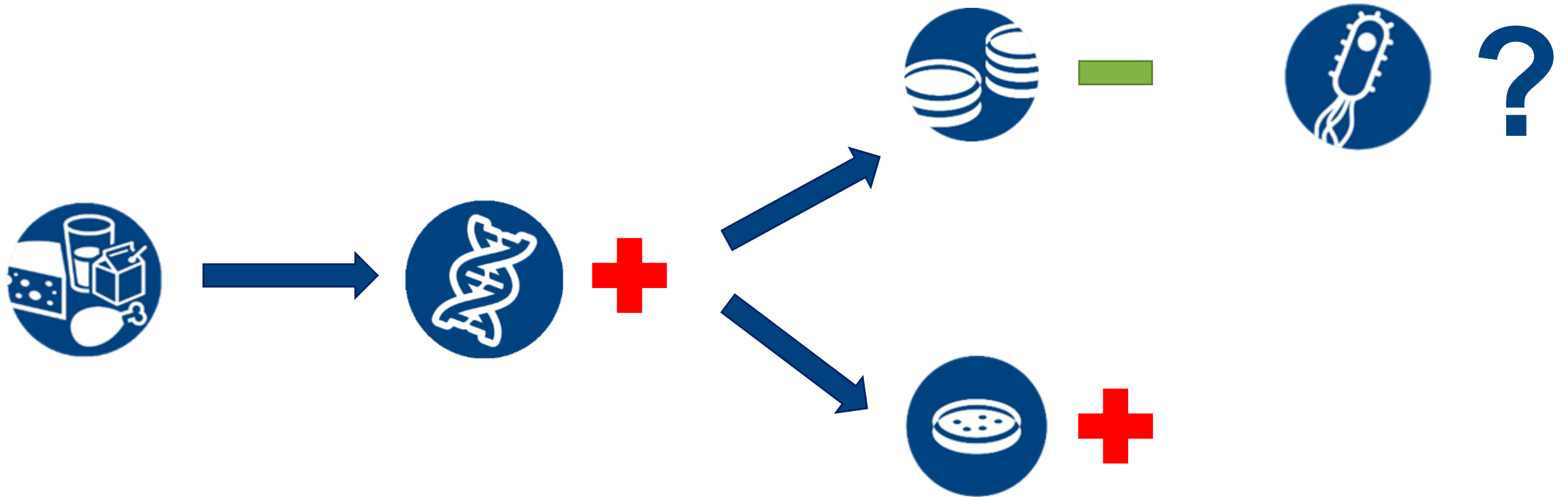
Na	Tar	V1	λ (nm)	CP	TM
sbx1/sbx	+	D2	640	27.11	65.7
eee	-	D2	705	33.62	
IC	✓	D2	705	33.62	61.75

Easy result interpretation

PERFORMANCE IMPROVEMENT

FALSE POSITIVES?

- PCR positive results not confirmed by cultural methods



FALSE POSITIVES?

- From 150 PCR positive stx results, 29 cultural isolated (19%). Verhaegen et al. 2016.

Table 2 | Frequency of STEC isolation by various methods in samples showing positive result for stx (either stx₁ or stx₂) by PCR methods.

	No of stx-positive samples by PCR	No of stx-positive samples by PCR with STEC isolation	Percentage of PCR stx-positive samples with STEC isolation (%)	References
Human stools (healthy slaughterhouse workers)	90	8	8.9	Hong et al., 2009
Humans stools (asymptomatic)	196	47	24	Stephan et al., 2000
Human stools (volunteers)	21	1	4.8	Urdahl et al., 2012
Human stools (hospital)	150	1	0.67	Urdahl et al., 2012
Human stools (hospital)	20*	10*	50	Buchan et al., 2013
Children stools (hospital)	21	5	24	Vallières et al., 2013
Children stools (hospital)	19	10	52.6	Pradel et al., 2000
Cattle feces	145	80	55.2	Fremaux et al., 2006
Cattle feces	154	67	43.5	Rogerie et al., 2001
Cattle feces	417	18	4.3	Hofer et al., 2012
Cattle feces	330	162	49.0	Pradel et al., 2000
Bovine hides	301	25	8.3	Monaghan et al., 2012
Bovine carcasses	122	5	4.1	Monaghan et al., 2012
Bovine carcasses	77	16	20.8	Breum and Boel, 2010
Bovine carcasses	91	16	17.6	Rogerie et al., 2001
Cattle environment	179	38	21.2	Fremaux et al., 2006
Beef meat	47	16	34.0	Pradel et al., 2000
Dairy buffalo (feces and milk)	56	20	35.7	Beraldo et al., 2014
Milk (bulk)	32	1	3.1	Trevisani et al., 2014
Milk (filters)	68	7	10.3	Trevisani et al., 2014
Cheese	60	5	8.3	Pradel et al., 2000
Healthy pigs feces	255	62	24.3	Meng et al., 2014
Swine feces	484	196	40.5	Fratamico et al., 2004
Meat products	36	8	22.2	Díaz-Sánchez et al., 2012

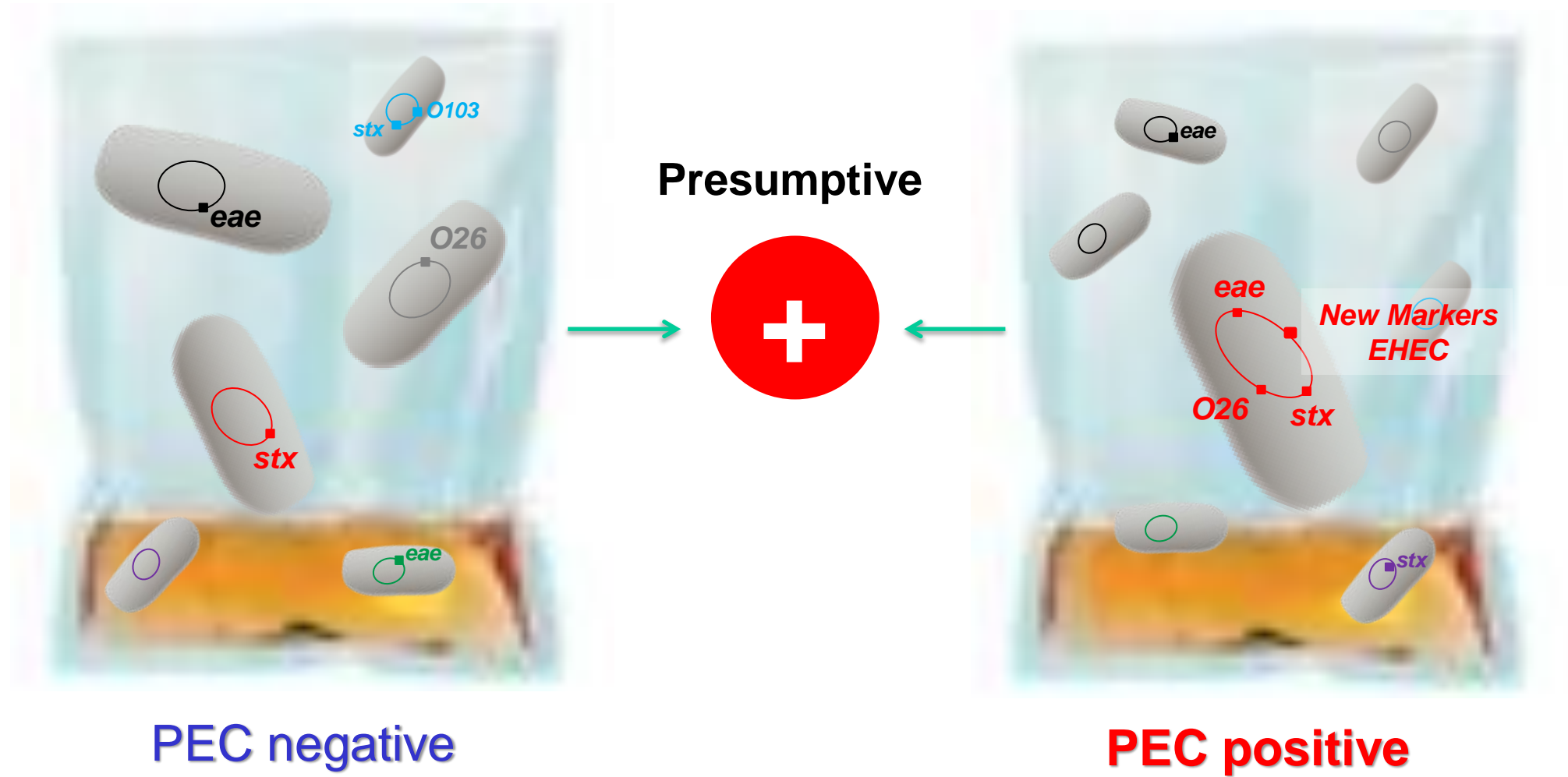
* Calculated from the % of positive samples.



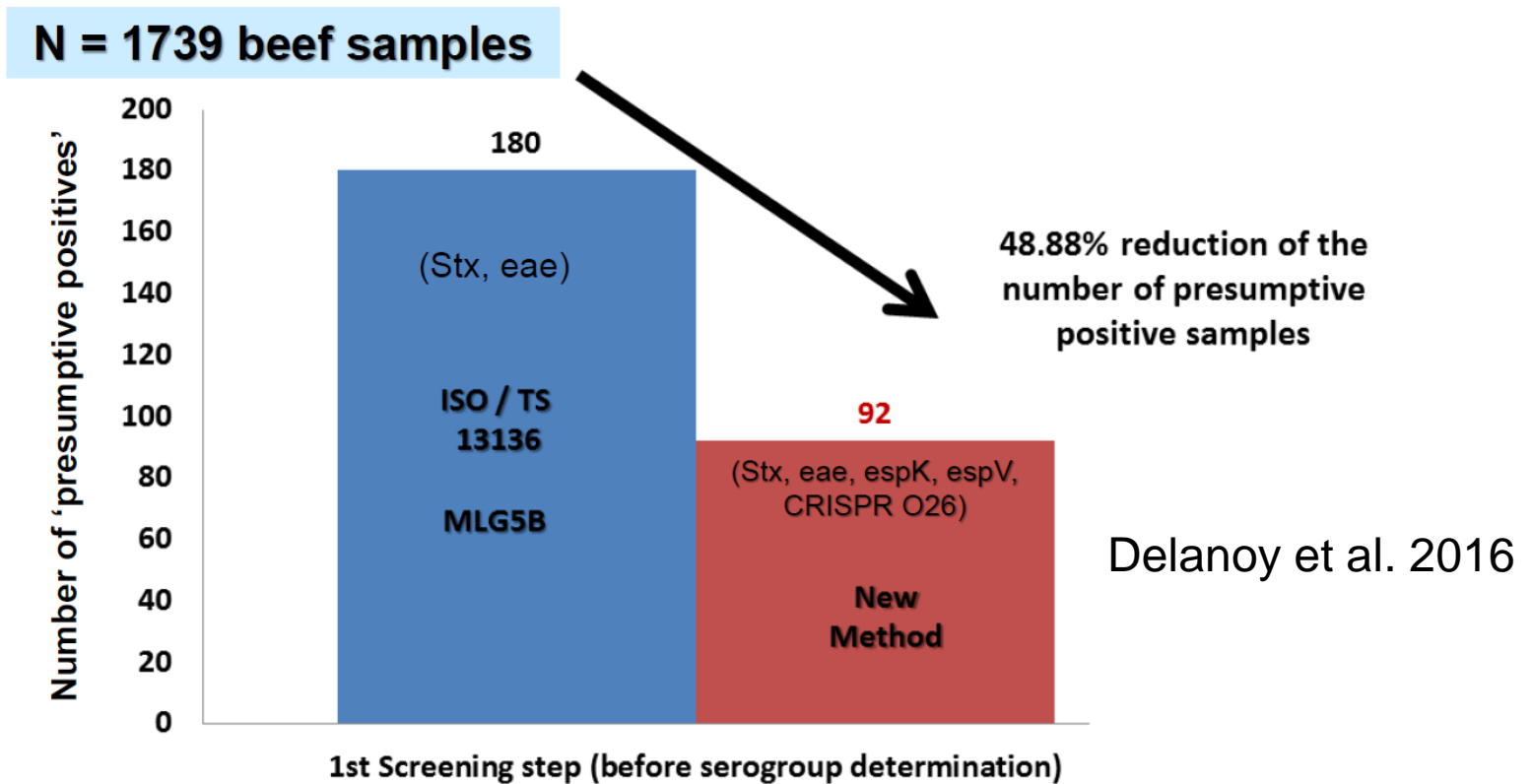
Revisiting the STEC Testing Approach: Using *espK* and *espV* to Make Enterohemorrhagic *Escherichia coli* (EHEC) Detection More Reliable in Beef

Sabine Delannoy¹, Byron D. Chaves², Sarah A. Ison², Hattie E. Webb², Lothar Beutin³, José Delaval⁴, Isabelle Billet⁵ and Patrick Fach^{1*}

NEW MARKERS



NEW MARKERS



- From 100 stx positive samples, 51 were positive for PEC and 4 positive by culture.
- From 82 stx positive samples, 47 were positive for PEC and 11 positive by culture (Four O103; Two O157:H7; Three O182; one O5; and one non-typable).

CHROMOGENIC MEDIA (AND IMMUNOSEPARATION)



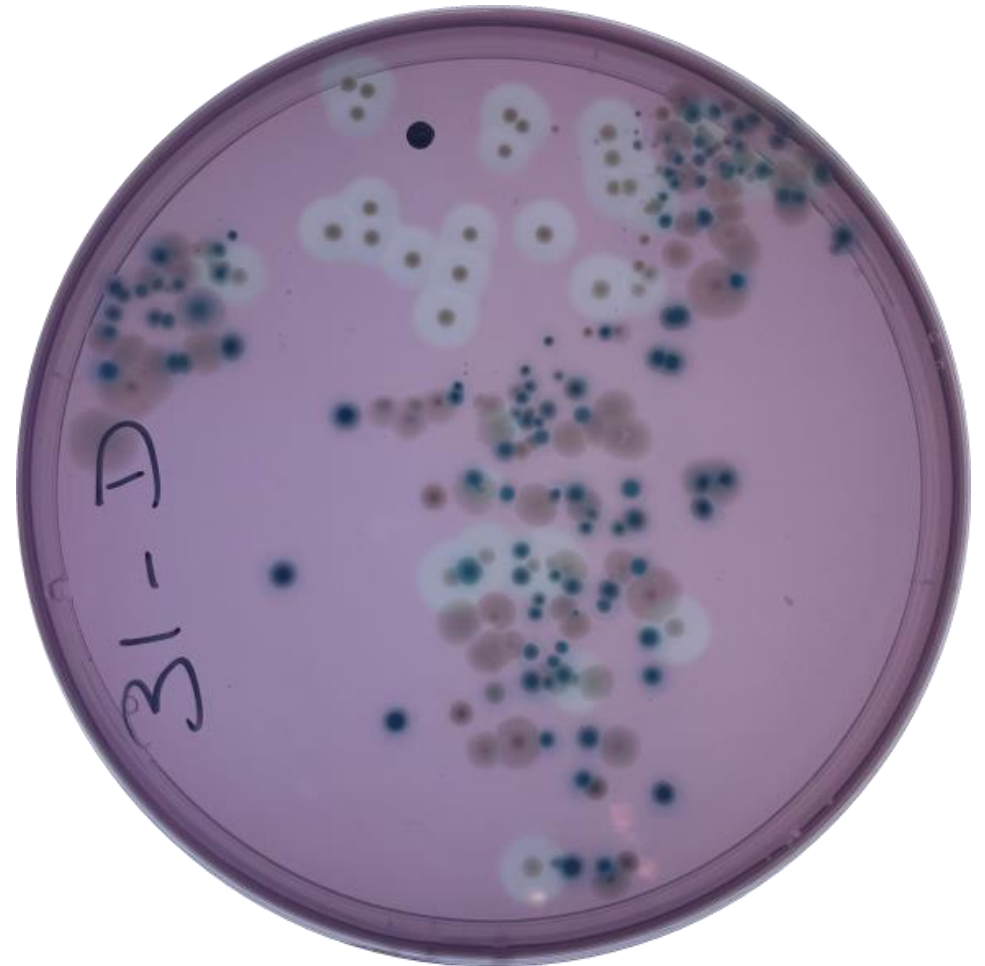
ChromID EHEC®

- Suspicion of O157**
Green to blue green colonies
- Suspicion of O121**
Violet, pink or magenta
- Suspicion of O111**
Violet grey to violet
- Suspicion of O45 or O145**
Violet
- Suspicion of O103**
Violet blue to violet
- Suspicion of O26**
Violet blue to violet

NEW AGAR FOR SIGA TOXIN DETECTION



STEC isolation in raw beef



STEC isolation in cheese from raw milk



PIONEERING DIAGNOSTICS