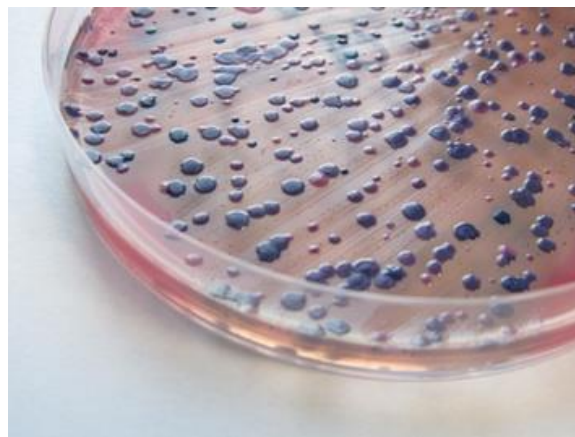


Successful isolations of STEC

**-NRL Finland, Helsinki,
November 2014 - October 2015**



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14th PT of EU-RL (sprouts)

Time: November 2014

Culture positive obtained from 7589 (1000 cfu/g level):

- Enrichment: BPW 37°C 20 h (storage in + 4C)
- Plating: Dilution series > plating of dilutions -1 to -7 > Harlequin™-CT-SMAC
- Unsuccessful attempts:
 - Direct plating (dilutions 0 to -3),
 - “Normal” IMS O104> plating on EHLy-, CT-SMAC, Harlequin™-CT-SMAC, CHROMagar STEC, RMAC and TBX

Trace-back investigation of STEC O145 infection

Time: March 2015

- Patient had eaten lamb casserole
- Trace-back to lamb farm and sampling

	PCR positives	Culture positives
Environmental samples	6 / 21	0 / 6
Feed samples	0 / 2	-
Fecal samples	10 / 10	2 / 10

Culture positives obtained:

- Enrichment: mTSB + 16 mg/l novobiocin, 41,5°C for 6 h
- Plating: IMS O145 > CHROMagar STEC and Harlequin™-CT–SMAC

⇒ All isolates recovered through O145 IMS

- Isolates were of serotype O128

15th PT of EU-RL (sprouts)

Time: April 2015

Culture positive obtained from 2184 (1000 cfu/g level):

- Enrichment: BPW 37°C 22 h (storage in – 20°C)
- Plating: Dilution series > plating of dilutions -5 to -8 > SHIBAM (STEC heart infusion washed blood agar with mitomycin-C)
- Second plating: Acid treatment after IMS O111 > SHIBAM and TBX

- Unsuccessful attempts:
 - Direct plating (dilutions 0 to -3),
 - “Normal” IMS O111 and dilutions -1 to -4 thereof
> plating on SHIBAM-, Harlequin™-CT–SMAC, CHROMagar STEC and TBX

Sprouting process / Own control sample, green lentil sprouts

Time: June 2015

■ ISO/TS 13136: 1/5 samples positive in PCR (*stx2* only, Cq 28)

Culture positive obtained from PCR positive:

■ Enrichment: BPW 37°C /18–24 h (storage in + 4°C)

■ Plating: Dilution series > plating of dilutions -5 to -8 > SHIBAM > purification on CHROMagar STEC and Harlequin™-CT–SMAC

■ Unsuccessful attempts:

■ Plating of dilutions -5 to -8 > CHROMagar STEC (only untypical growth)

■ No attempts of IMS since the lack of top-8 serotype signal

Project on raw milk hygiene / longitudinal monitoring of three farms

Time: February 2014 - May 2015

■ Sampling of raw milk, milk filters, feces and environment

	PCR positives	Culture positives (serotypes)
Raw milk	52 / 789	1 / 789 (O121)
Milk filters	233 / 631	18 / 632 (O157, O26, NT)*

*O157 (12 strains), O26 (5 strains), NT (1 strain)

Culture positives obtained:

■ Enrichment: mTSB + 12 mg/l acriflavin 37°C/18–24 h

■ Plating: Direct culture and IMS O157

➤ Harlequin™-CT–SMAC and CHROMagar STEC

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Summary

- IMS (as described in ISO 16654) often not successful (to non-O157 STEC in food with high background flora?)
- Dilution series after enrichment recommendable
 - Normal plating set for us: SHIBAM-, Harlequin™-CT–SMAC, CHROMagar STEC and TBX
- In addition to dilution, acid treatment after IMS O111 was successful in PT 15