Persistence of STEC in grain, flour, and raw dough

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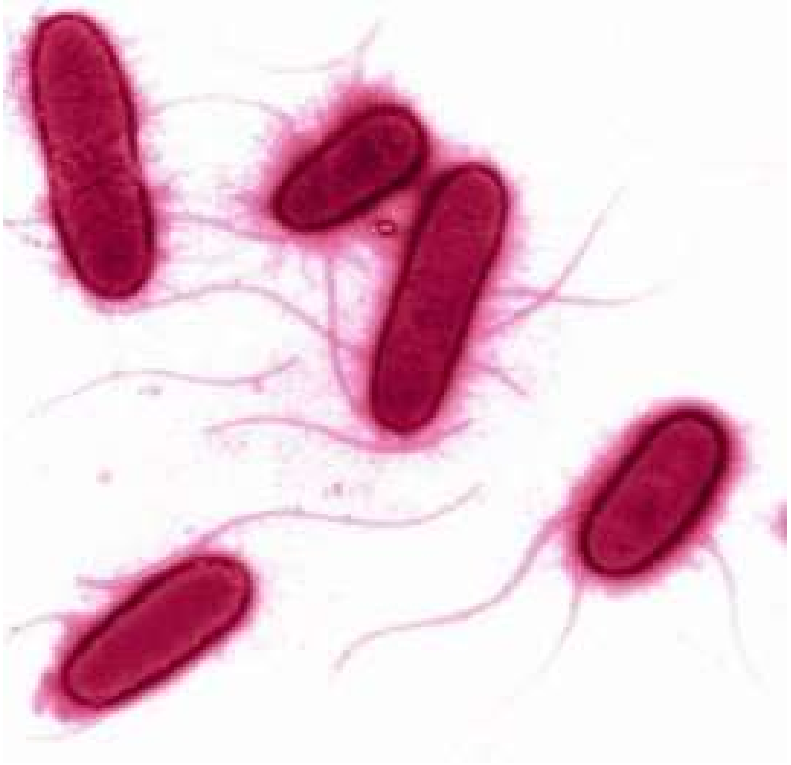
Shiga Toxin producing *E. coli* (STEC)

- *Enterobacteriaceae*
- pathogenic *E. coli*
- causative agent of the hemolytic uremic syndrom (HUS: kidney damage, inflamation and failure)
- STX: type AB enterotoxin, burst released, glycosidase, targets 23S rRNA
- Several variants of STX known, *stx1* and *stx2* are used for detection (ISO 13136)
- Additional virulence factor: intimin adherence protein, encoded by *eae* gene (ISO 13136)
- CH, HyV: must not be present in 25 g of sprouts (O157, O26, O111, O103, O145 and O104:H4), no regulation for any other food category
- Key-enzymes for LPS O-antigen synthesis need to be detected (ISO 13136, typing needed)
- Reservoir: productive lifestock (mainly cattle) and wild animals
- Route of transmission: fecal oral

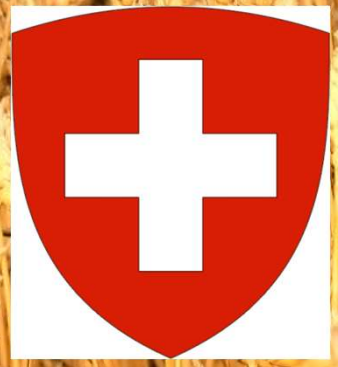


Outbreaks / Prevalence Flour

- Outbreaks of *E. coli* O121 from contaminated flour and semi-finished doughs in the US and Canada in 2016 (Crowe et al. 2017; Gieraltowski et al. 2017; Morton et al. 2017)
- Sampling of Swiss and German wheat and spelt flours yielded viable STEC isolates in ca. 10 % of all samples analyzed (Mäde et al. 2017; Kindle et al. 2019; Boss and Hummerjohann 2019)

Aim of this study

- BLV project 4.18.02
- Determine the persistence of STEC in wheat grain, flour, and raw dough (commercial products)
- Determine the prevalence of STEC in wheat grains
- Develop a quantitative detection method



Persistance of STEC in wheat grain, flour, …

- Selection of strains is important
- Detection of the bacteria during the challenge test
- Chromogenic agar cannot be applied (β-glucuronidase negative (TBX) and/or *terD* negative (tellurite resistance, ChromagarTM STEC))
- Strains were selected/adapted to be Streptomycin resistant
- Growth kinetics were compared wt vs mutant

Species	Strain	Serotype	*stx*	Str^R	Origin
E. coli	222	O28	negative	yes	R. Stephan, UZH
E. coli	584	O157	negative	yes	R. Stephan, UZH
E. coli	M31c	unknown	negative	adapted	R. Boss, BLV, flour isolate
E. coli	M66	unknown	negative	adapted	R. Boss, BLV, flour isolate
E. coli	M87	unknown	negative	adapted	R. Boss, BLV, flour isolate

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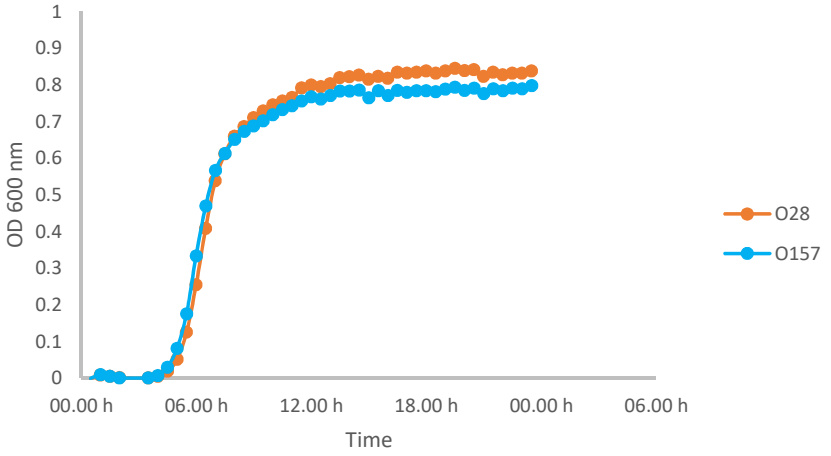
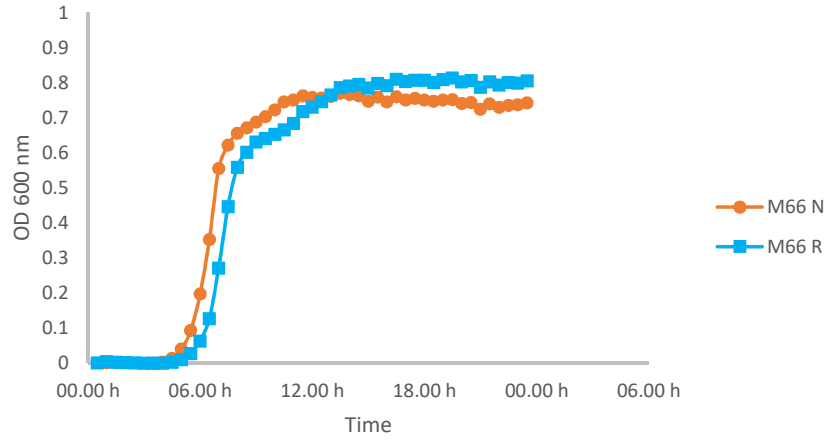




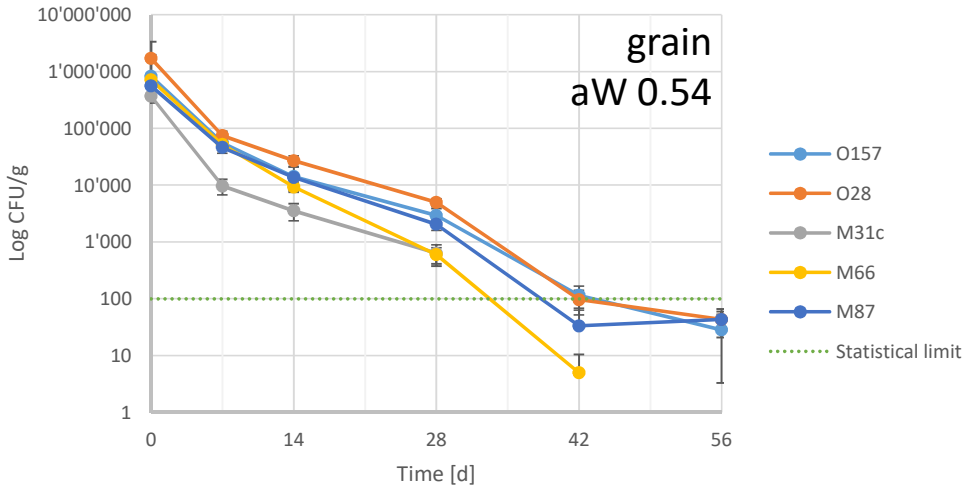
Hypothesis

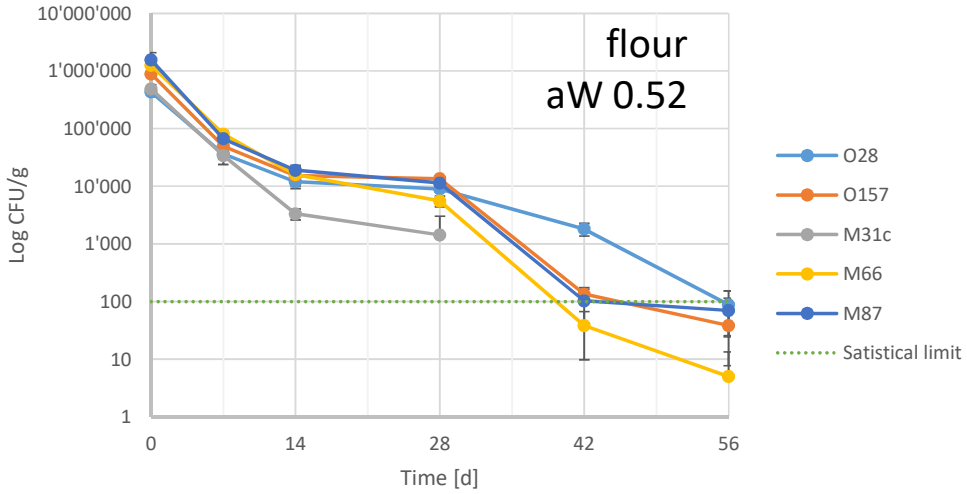
- Wheat flour: aW 0.52
- no growth
- likely a reduction of cfu over time
- likely persistence over time

- Wheat grain: aW 0.54

Persistance in wheat grain and flour

- high starting inoculum 10^6 cfu/g
- Samples stored at RT
- ~ 1 log reduction after 7 days
- ~ 2 log reduction after 14 days
- ~ 4 log reduction after 42 days
- viable bacteria were still detectable after 56 days



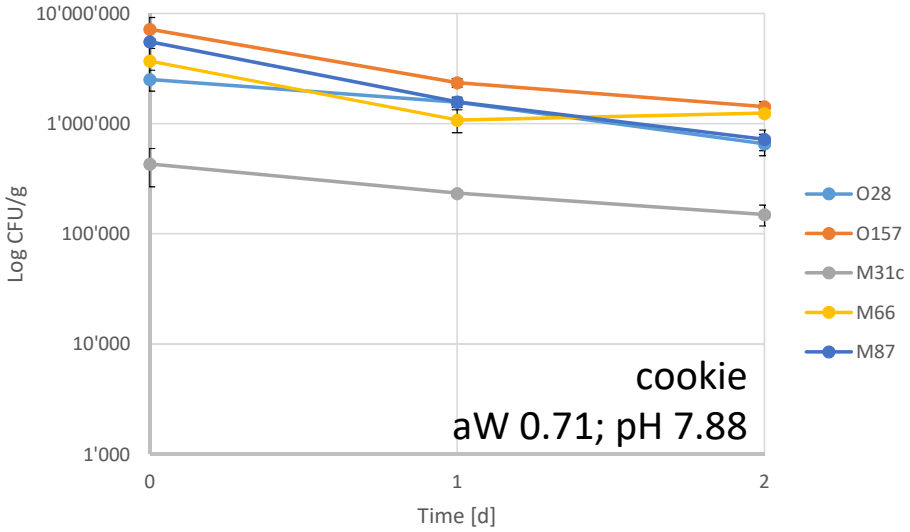


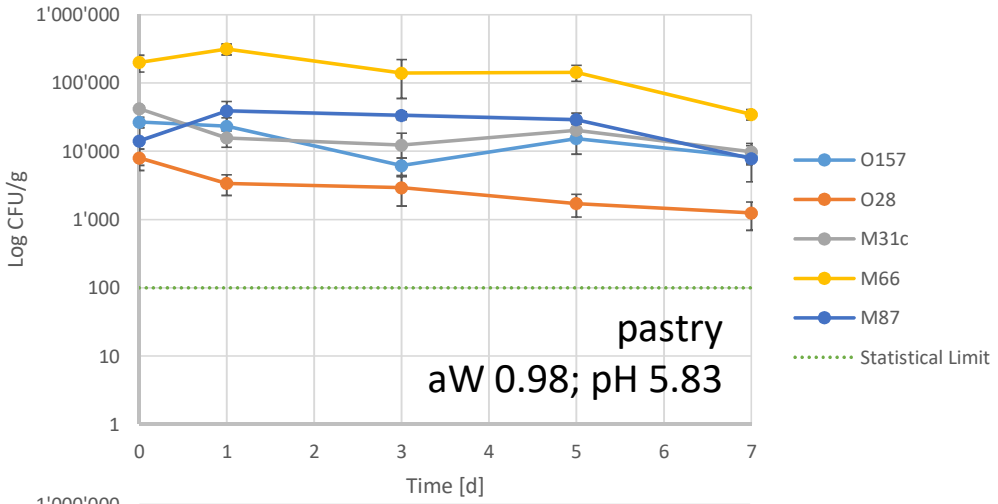
Hypothesis

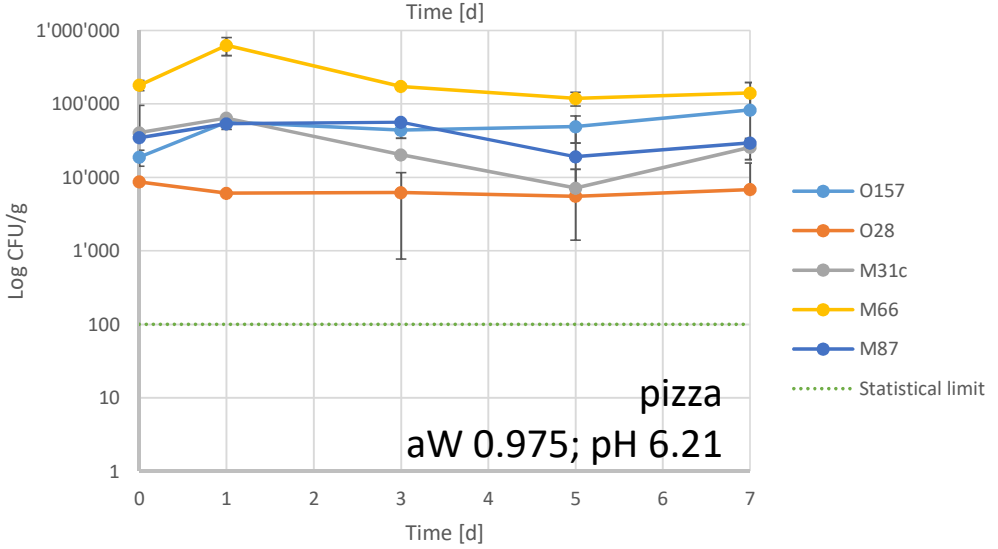
- Pastry dough: aW 0.98; pH 5.83
- Pizza dough: aW 0.975; pH 6.21
- Cookie dough: aW 0.71; pH 7.88
- good growth

Persistance in raw dough

- Starting inoculum 10^5 cfu/g (pastry and pizza) and 10^7 cfu/g (cookie)
- stored at RT
- stable counts in pastry and pizza dough
- slight reduction of ~ 0.5 log in cookie dough







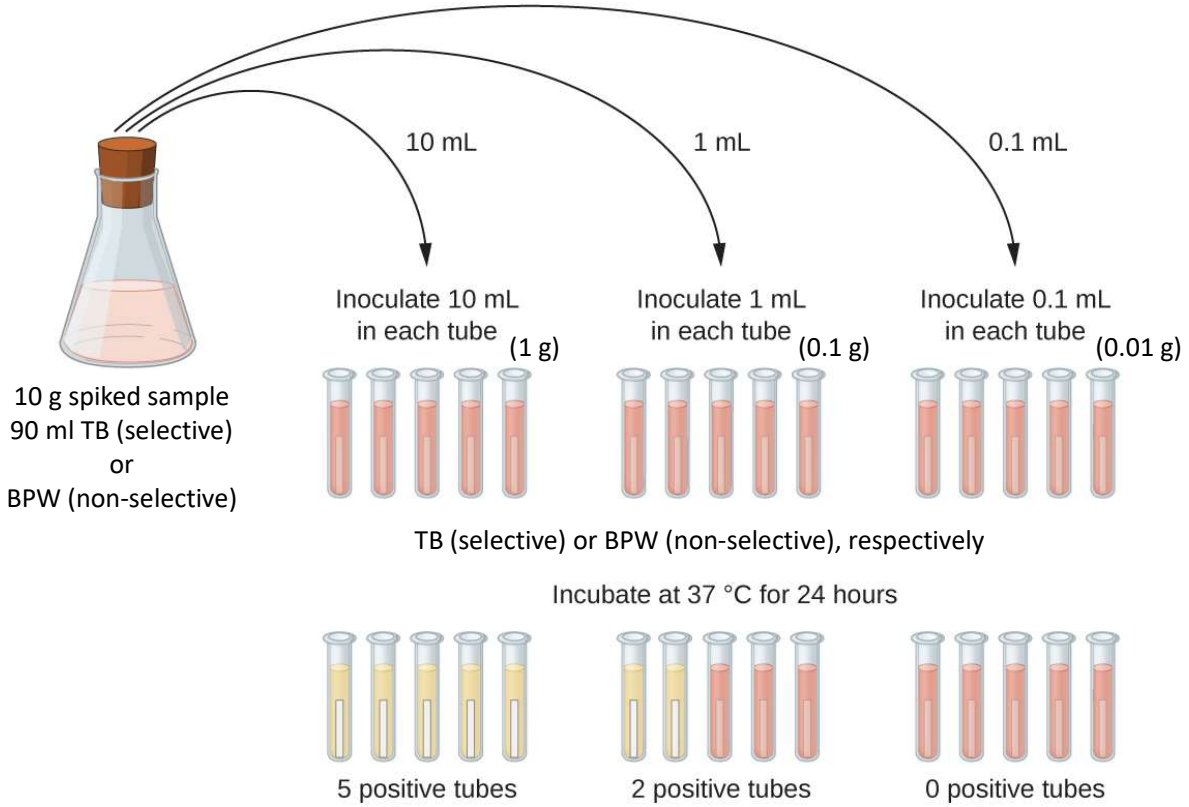
Prevalence of STEC in grain

- Wheat grain was collected during the harvest season 2019
- 100 whole wheat grain samples (25 g) were analysed for the presence of STECs
- in addition 20 soaked grain samples (25 g) were analysed for the presence of STECs
- Non selective enrichment in BPW, 24 h, 37°C
- Selective isolation on TBX, 24 h, 44°C
- Bacterial matter was elutet and subjected to qPCR targeting *eae*, *stx1*, and *stx2* genes

type of grain	samles	*E. coli*	*eae*	*stx1*	*stx2*
after harvest, dried	100	40/100	0/100	0/100	0/100
soaked before milling	20	2/20	0/20	0/20	0/20

quantitiative detection of STEC from grain or flour

- viable cell counts are rather low
- sensitive method is needed
- complex matrix
- MPN combined with qPCR
- *E. coli* 584 (O157) was used
- qPCR targeted *rfbE* gene (O-antigen Synthesis)
- Inoculum of 10^4, 1000, 100, and 10 cfu/g
- Samples/inocula were analyzed in triplicates
- Each MPN tube was analyzed in duplicates
- As a control cfu/g were also determined by standard plating



qPCR on each tube

https://microbeonline.com/probable-number-mpn-test-principle-procedure-results/

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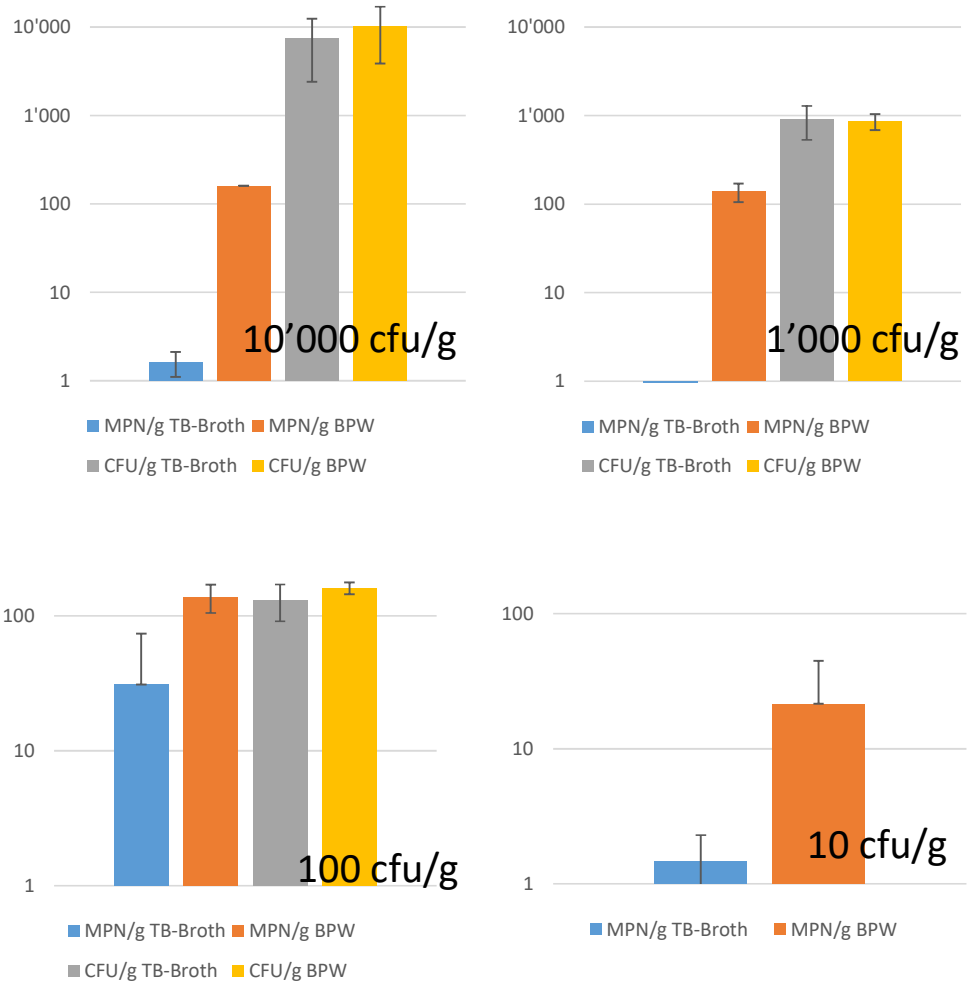
- As a control cfu/g were also determined by standard plating
- MPN using TB underestimates viable cell counts



Summary and conclusion

- *E. coli* O28 and O157 and other serotypes seem to survive in flour and grain for 56 days (8 weeks) or even longer
- Forghani et al. 2018 reported survival, e.g. presence of viable *E. coli* O26, O103, O111, and O157, even after 280 days (40 weeks)
- Forghani et al. 2019 reported presence of viable *E. coli* O45, O121, O145, and *Salmonella* after 168 days (24 weeks)
- Gill et al. 2019 isolated viable *E. coli* O121 after 2 years of wheat storage (outbreak strain, Canada 2016)
- Moreover the bacteria survived heat treatment of wheat (70°C)
- Michael et al. 2019 demonstrated that *E. coli* O121 is completely eradicated if contaminated flour is used to prepare and bake muffins
- Gill et al. 2019 further demonstrated that the *E. coli* O121 outbreak strain was present in naturally contaminated flour at 0.15 to 0.43 MPN/100 g
- “There was no evidence of higher levels of organisms associated with fecal contamination in the recalled flour.”

Summary and conclusion

- STEC are likely transmitted to grain on the field (fecal contamination)
- In flour viable cell counts are likely very low
- However, the bacteria persist very long (2 years)
- Infectious dose is very low (10 cfu)
- Backing inactivates STECs
- Qualitative detection methods are established (ISO13136)
- Quantitative detection should be performed using MPN-qPCR (sample size, complex matrix)
- Instead of a selective enrichment BPW, e.g. a non-selective enrichment, should be applied

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