# A diagnostic screening algorithm to asses the public health risk of STEC.

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## Introduction

Shiga toxin-producing *Escherichia coli* (STEC) poses a serious public health concern because of its ability to cause outbreaks, hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS). In particular, STEC strains belonging to serotypes O26, O103, O111, O121, O145 and O157 are known causative agents of outbreaks and serious illness. This study describes a rapid screening algorithm to discriminate STEC infections associated with public health risk from less virulent ones.

## Materials and Methods

- A total of 5022 stool samples from patients with presumed infectious gastroenteritis were prospectively screened using real-time multiplex PCR (mPCR). Subsequently, PCR positive stool specimens were cultured directly (SMAC/CHROMagar STEC) and after enrichment (>16hrs) (Figure 1).
- Simultaneously, enriched BRILA broth was used for DNA isolation. Real-time mPCRs were performed targeting serotype specific genes and additional virulence factors. Suspicious CHROMagar colonies (n=5 per sample) were also tested by mPCRs. PCR results were used for presumptive seropathotype (SPT) classification (Karmali *et al.* JCM 2003) and to assess the presence of viable STEC.
- Furthermore, culture isolates were characterized using sero-/genotyping (RIVM) and DNA microarray analysis (UMCG).

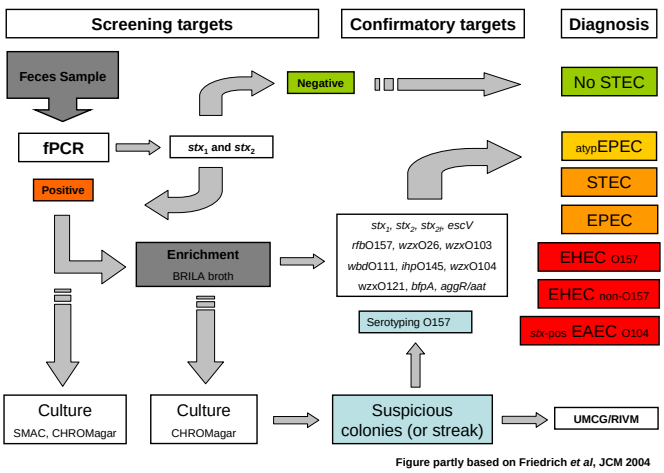


Figure partly based on Friedrich *et al*, JCM 2004

**Figure 1.** STEC diagnostic screening algorithm.

## Results

- In total, 90/5022 (1.8%) stool samples were PCR positive for $stx_1$/$stx_2$. The algorithm was applied to 73 samples (70 patients).
- Direct PCR for $stx_1$/$stx_2$ was confirmed by "enriched" PCR for 65 samples (89%). In eight samples (11%) no viable STEC could be detected (all $C_T \geq 34$ in direct PCR).
- The $stx_1$/$stx_2$ $\Delta C_T$ values of enriched vs direct samples ($C_T$ BRILA – $C_T$ direct) ranged from +9 to -21. In 55 (85%) enriched samples the $\Delta C_T$ value was $\leq 0$.

## Results continued

- In total, 31/73 samples were confirmed by culture (42%).
- The diagnostic screening algorithm (presumptively) classified $stx_1$/$stx_2$-pos samples in the following seropathotype (SPT) groups:

***SPT A (n=2, 3%)***

| BRILA PCR | | | | | Culture | | | |
|---|---|---|---|---|---|---|---|---|
| *stx1* | *stx2* | *escV* | O-type/virulence | Ct | SMAC | CHROM | Serotype | Diagnosis |
| 15 | 17 | 16 | O157 | 18 | POS | POS | O157:H7 | EHEC O157 |
| NEG | 29 | 27 | O157/EAEC | 31/19 | NEG | NEG | | EHEC O157/EAEC |

***SPT B (n=8, 11%)***

| BRILA PCR | | | | | Culture | | | |
|---|---|---|---|---|---|---|---|---|
| *stx1* | *stx2* | *escV* | O-type/virulence | Ct | SMAC | CHROM | Serotype | Diagnosis |
| 15 | NEG | 16 | O26 | 17 | POS | POS | O26:H11 | EHEC O26 stx1 |
| 26 | 28 | 26 | O145 | 27 | NEG | POS | O145:H- | EHEC O145 |
| NEG | 24 | 25 | O157/O26/O145 | 31/29/24 | NEG | NEG | | EHEC O145/O26/O157 |
| 15 | NEG | 16 | O26 | 17 | POS | POS | O26:H11 | EHEC O26 stx1 |
| NEG | 31 | 22 | O145/O121/O26 | 22/31/38 | NEG | STREAK | O26 | EHEC O26 stx2 |
| NEG | 32 | 29 | O26 | 32 | NEG | POS | O26:H11 | EHEC O26 stx2 |
| 18 | 21 | 19 | O26 | 20 | POS | POS | O26:H11 | EHEC O26 |
| NEG | 18 | 16 | O145 | 16 | POS | POS | O145:H- | EHEC O145 |

***SPT C (n=10, 14%)***

| BRILA PCR | | | | | Culture | | | |
|---|---|---|---|---|---|---|---|---|
| *stx1* | *stx2* | *escV* | O-type/virulence | Ct | SMAC | CHROM | Serotype | Diagnosis |
| 21 | NEG | 22 | NEG | NEG | NEG | NEG | | EHEC stx1 |
| 15 | 19 | 17 | NEG | NEG | POS | NEG | O165:H- | EHEC O165 stx1/stx2 |
| NEG | 24 | 20 | NEG | NEG | NEG | POS | O101:H- | EHEC O101 stx2 |
| 16 | NEG | 21 | NEG | NEG | NEG | NEG | | EHEC stx1 |
| 21 | NEG | 27 | NEG | NEG | NEG | NEG | | EHEC stx1 |
| 19 | NEG | 20 | EAEC | 25 | NEG | NEG | | EHEC stx1/EAEC |
| NEG | 25 | 17 | NEG | NEG | NEG | POS | O182:H25 | EHEC O182 stx2 |
| NEG | 26 | 23 | NEG | NEG | NEG | NEG | | EHEC stx2 |
| 31 | 32 | 31 | NEG | NEG | NEG | POS | O182:H25 | EHEC O182 stx1/stx2 |
| NEG | 23 | 19 | NEG | NEG | POS | NEG | ONT:H31 | EHEC ONT stx2 |

***SPT D (n=45, 62%)***

- Of which: *stx1* (n=24), *stx2* (n=8), *stx2f* (n=9) and *stx1/stx2* (n=4). A total of 17 samples were confirmed by culture (only 3 by CHROM agar). Most identified O-type in SPT D group was: O91 (5x).
- Co-infections were identified in 31/73 samples (42%), especially in the presumptively identified SPT D group (n=22/45, 49%).
- DNA microarray analysis confirmed the feno-/genotypic O- and H-serotyping results in the majority (76% and 68%, respectively) of isolates (n=27). In addition, characterization also identified an EPEC (O88) and EAEC (O104:H4). Finally, no ESBL genes were found in the isolates.

## Conclusions

- **The diagnostic algorithm enables fast discrimination of virulent STEC associated with outbreaks (SPT A and B) and HUS (SPT A, B and C) from less virulent STEC (SPT D).**
- **Compared to routine SMAC, the CHROMagar STEC improved the culture yield with additional identification of non-O157 isolates.**
- **The algorithm enables the detection of viable STEC in stool samples.**
- **DNA microanalysis appears to be a useful typing method for *E. coli* isolates and can be used for fast detection of virulence- and resistance genes + O:H serogenotyping.**

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