R-Biopharm AG

Evaluation and comparison of the RIDA®GENE EHEC/EPEC real-time PCR for the detection of STEC (Shiga toxin-producing *E. coli*) from human feces, liquid enrichment broth and agar plate

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Background

Shiga toxin-producing *E. coli* (STEC) also called verotoxin-producing *E.coli* (VTEC), produce one or more potent cytotoxins and are capable of causing watery diarrhea, bloody diarrhea, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura and death. STEC strains that cause bloody diarrhea are also called enterohemorrhagic *E. coli* (EHEC).

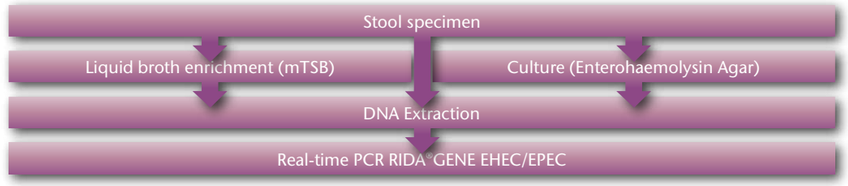
The morbidity and mortality rates associated with STEC outbreaks have highlighted the threat to public health. The CDC estimates that *E. coli* O157:H7 causes more than 20.000 infections and 250 deaths in the United States each year. During the EHEC outbreak in Germany 2011 more than 4300 infections and 49 deaths were reported. Conventional tests for STEC require 24 to 36 h culture or enrichment broth of the bacteria for detection. The RIDA®GENE EHEC/EPEC real-time PCR assay allows in less than 2 hours the direct detection of STEC in stool specimens. A rapid and early diagnosis of STEC allows appropriate patient management, prevents antimicrobial treatment that may increase the risk of HUS and avoid spread of the infection. The aim of the study was to evaluate and compare the performance of the RIDA®GENE EHEC/EPEC for the detection of STEC from stool specimen, liquid enrichment broth culture (EB) and agar plate.

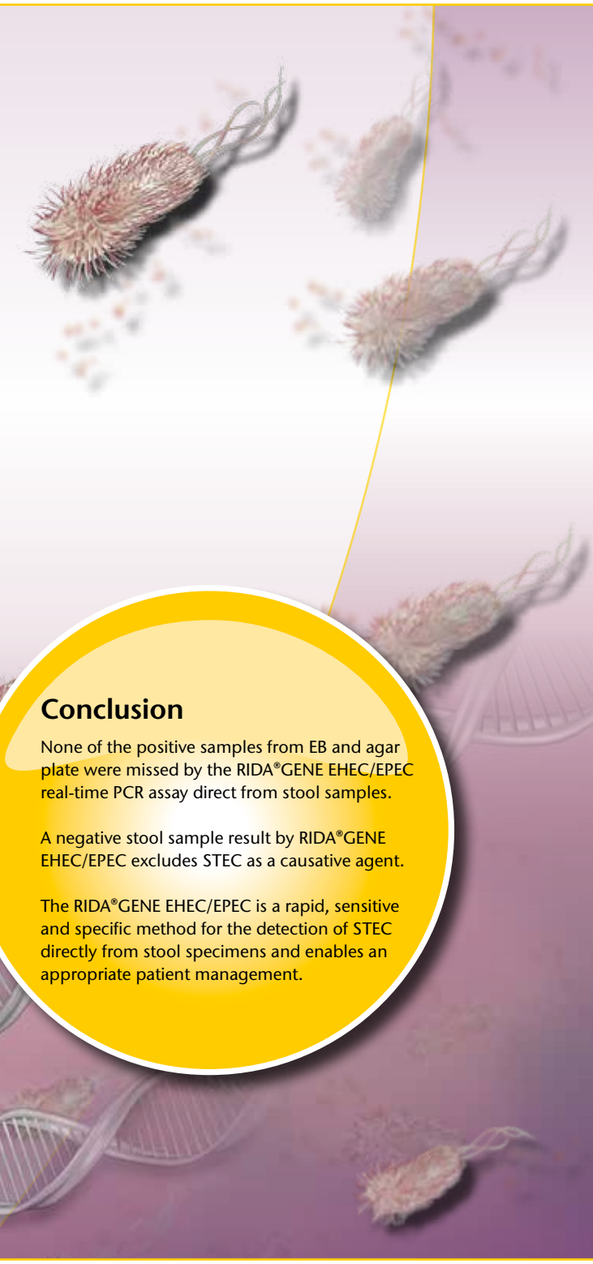
Picture 1: RIDA®GENE EHEC/EPEC multiplex real-time PCR

Methods

A total of 90 stool samples from patients with signs and symptoms of acute gastroenteritis suspected of being caused by STEC were enriched in liquid broth (mTSB; R-Biopharm AG) and cultured on enterohaemolysin agar. Positive colonies (1 loop) were resuspended in 1ml water, incubated at 95°C for 5 min and homogenized.
The homogenized samples were tested by RIDA®GENE EHEC/EPEC, a qualitative real-time PCR assay that targets the virulence-factor genes stx1/sxt2, eae and ipaH with fluorogenic target-specific hydrolysis probes. An Internal Control DNA (ICD), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control ensures reliable results. Stool specimen and liquid broth of all samples were also tested by RIDA®GENE EHEC/EPEC. Therefore the stool sample was diluted 1:3 with water, vortexed and centrifuged at 3.000 rpm for 30 sec. The stool sample and liquid broth were extracted according to the High Pure PCR template preparation kit instruction (Roche). Amplifications were performed using the LightCycler® 480II (Roche).

Fig.1: Study design





Results

Among the 90 stool samples 17 samples (19 %) were positive for STEC from liquid enrichment broth culture and agar plate and 18 samples (20 %) were positive direct from stool, respectively. The average Ct value of stool samples compared to EB samples was 6 Ct higher and 10 Ct higher compared to agar plate samples, respectively. Some of the stool samples showed almost no difference in the Ct value compared to EB and/or agar plate samples.

Fig. 2: Summary of STEC results from stool, liquid enrichment broth and agar plate

STEC positive samples	Ct value		
	Stool	Enterohaemolysin agar	LIQUID ENRICHMENT BROTH (mTSB)
1	31.83	26.1	17.61
2	39.87	0	0
3	35.32	27.54	28.61
4	29.67	28.09	19.93
5	27.81	26.73	26.01
6	40.52	28.02	24.16
7	32.1	26.32	17.91
8	25.61	27.39	21.75
9	35.96	23.43	21.81
10	28.42	27.88	21.17
11	37.24	27.24	27.93
12	30.89	29.85	14.01
13	24.85	20.84	16.89
14	27.28	21.21	17.16
15	35.87	24.68	18.75
16	28.19	19.92	19.85
17	30.37	19.87	23.39
18	28.95	23.69	16.9
Mean	**31.71**	**23.82**	**19.66**
Mean (w/o # 2)	**31.23**	**25.22**	**20.81**
NTC	0	0	0
PTC	29.79	30.99	30.8

Fig. 3: Comparison of Ct-values for STEC samples from stool, liquid enrichment broth and agar plate

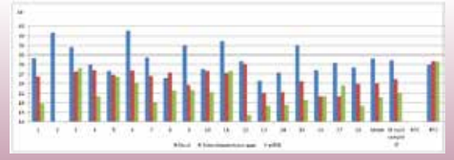


Fig. 4: Example of STEC runs from stool on the LC480II

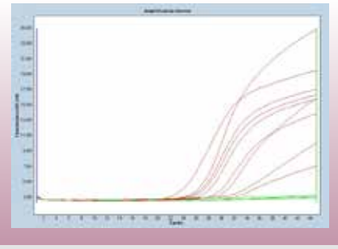


Fig. 5: Example of STEC runs from liquid enrichment broth (mTSB) on the LC480II

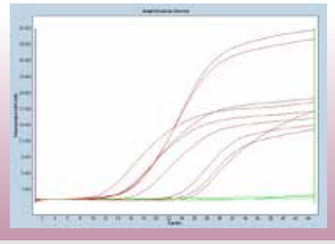
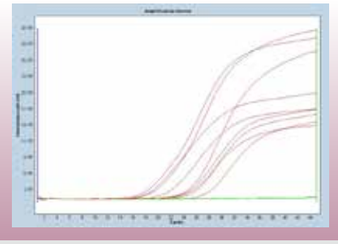


Fig. 6: Example of STEC runs from agar plate on the LC480II



Conclusion

None of the positive samples from EB and agar plate were missed by the RIDA®GENE EHEC/EPEC real-time PCR assay direct from stool samples.

A negative stool sample result by RIDA®GENE EHEC/EPEC excludes STEC as a causative agent.

The RIDA®GENE EHEC/EPEC is a rapid, sensitive and specific method for the detection of STEC directly from stool specimens and enables an appropriate patient management.

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