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Paper Poster Session
Diagnosing gastrointestinal tract infection

Evaluation of a real-time PCR assay for the detection of enteroaggregative E. coli directly in stool specimens

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Background: Enteroaggregative E. coli (EAEC) is increasingly recognized as a cause of persistent diarrhoea in both children and adults and several outbreaks have been reported. However the use of EAEC diagnostics in laboratory test panels is not quite common, especially in developed countries. We evaluated a Real-Time PCR method, the RIDA®GENE EAEC for the direct detection of EAEC in stool specimens. In addition we also determined the prevalence of this pathogenic agent in stool specimens of symptomatic patients with acute gastroenteritis but negative for other diarrheal pathogens.

Material/methods: 168 stool specimens of children or returning travelers, negative for other pathogens, with signs and symptoms of acute gastroenteritis were analyzed with the RG EAEC assay. The results of the RG EAEC assay were compared to specific PCR and sequencing methods performed from colony material and directly from stool. The extraction procedure for the real-time PCR assays was performed with the EasyMAG system (bioMérieux) according to the manufacturer's instructions. The real-time RG EAEC PCR assay was performed on the LightCycler480II. RG EAEC PCR detects the presence of the *aat*A and the *agg*R-gene specific for EAEC (465nm-510nm). The success of the PCR reaction and extraction is monitored by an extraction control. For a total volume of 20 µl, 19.9 µl of Reaction Mix and 0.1 µl of Taq-Polymerase are combined. 5µl of the target DNA are used for each PCR reaction. The assay was performed according to manufacturer´s instructions.

Results: For the detection of EAEC, the RG EAEC-PCR was performed directly from stool and from enrichment culture. Of 168 stool specimens from children or returning travelers with symptoms of acute gastroenteritis tested for EAEC, 21 were positive by PCR (12.5%). The 21 positive specimens included 14 patients with travel history, among these, 3 were children. All 7 patients with no travel report were children. The EAEC-PCR results were confirmed by the German Reference Center (RKI, Wernigerode, Germany) or by sequencing. One EAEC negative specimen showed inhibition.

Conclusions: The RIDA®GENE EAEC Real-Time PCR showed sensitive and specific results for the detection of EAEC in stool specimens. In this study a high frequency (12.5%) of EAEC especially in patients with risk factors was determined.