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STEC Screening and STEC Identification using real-time PCR (#582)

Ivo Meier-Wiedenbach, Astrid Grönewald, Cordt Grönewald, Kornelia Berghof-Jäger

BIOTECON Diagnostics, Potsdam, Germany

Introduction

Every year, food-borne illnesses caused by Shiga toxin-producing *Escherichia coli* (STEC) claim many lives worldwide. STEC are most commonly transmitted through raw ground beef, raw or inadequately pasteurized milk, sprouts and vegetables. Most infections are caused by *E. coli* serotype O157 and a number of non-O157 *E. coli* serotypes (e.g., "Big Six"). In 2011, *E. coli* O104:H4, not a member of the "Big Six", caused a serious outbreak in Germany. ISO/TS 13136 requires screening for the virulence factors *stx1*, *stx2*, and the intimin *eae*, following identification of five serotypes (O26, O103, O111, O145 and O157). Besides O157, the US focuses on the "Big 6" (O26, O45, O103, O111, O121 and O145). Moreover, in the European Union there was an extension of regulation (EC) 2073/2005, which additionally requires testing for O104 serogroup.

Methods

BIOTECON Diagnostics has developed two real-time PCR kits that enable the easy screening for STEC and the subsequent identification of the most important eight serotypes (O26, O45, O103, O104, O111, O121, O145 and O157) in less than 24 hours. The foodproof® STEC Screening LyoKit detects *stx1*,

stx2, *eae*, and the internal amplification control in one single PCR reaction. Following screening, the most important eight serotypes can be identified by melting curve analysis in just one additional PCR reaction using the foodproof® STEC Identification LyoKit.

Results

During validation, all tested 81 STEC strains comprising at least 13 different O-serotypes STEC have been detected and correctly identified. The limit of detection of pure DNA is at least 20 genome equivalents for all eight target serotypes. The assay is based on ISO/TS 13136, but it was further expanded to detect all known variants of the *stx*-genes. In particular, *stx2f* is missing from the ISO method, but can be easily detected with the screening assay.

Conclusion

For convenience, safety, and easy storage the reaction mixes in the foodproof® LyoKits are pre-filled and lyophilized, so that the sample DNA can be added directly to the reaction tube. Thus, these kits allow an easy and convenient screening and identification of the "Big Six", O157, and O104 in less than 24 hours using real-time PCR technology.

Notes