Detection of Escherichia coli virulence-associated genes with Kylt® Multiplex Real-Time PCR

Intestinal pathogenic Escherichia coli are the most common cause of diarrhoea and septicaemia in piglets, cattle and small ruminants, resulting in significant economic losses.

While most E. coli are harmless commensals which are part of the natural gastrointestinal flora of warm-blooded animals, some subsets, like the intestinal pathogenic E. coli, have acquired specific virulence-associated genes which enable them to be pathogenic in target hosts.

Virulence factors, responsible for pathogenicity, are fimbriae and adhesins as well as different toxins.

Detection and differentiation of virulence-associated genes of E. coli by using Kylt® Multiplex Real-Time PCR systems enables determination of potential virulence of E. coli isolates and allows to distinguish between different pathotypes (Table 1). Since these pathotypes cause different symptoms in the host, the analysis of E. coli isolates with Kylt® Multiplex Real-Time PCRs contributes to a differentiated diagnosis.

Table 1: Virulence factors for identification of E. coli pathotypes

Pathotypes	Fimbriae and adhesins	Toxins	Kylt® Multiplex Real-Time PCR Kits covering fimbriae/adhesins and/or toxins
Enteropathogenic E. coli (EPEC)	Intimin (eae)	EAST	Kylt® E. coli eae, EAST
Enterotoxigenic E. coli (ETEC)	F4, F5, F6, F17, F18, F41, FimA/FimH, AIDA, paa	STI, STII, EAST, LTI, Stx2e,	Kylt® E. coli F4,F5,F6 Kylt® E. coli FimA, FimH, F41 Kylt® E. coli Sta, Stb, LT Kylt® E. coli F18, F41, Stx2e Kylt® E. coli F5, F17, F41
Edema disease E. coli (EDEC)	F18, AIDA	Stx2e, EAST	Kylt® E. coli F18, F41, Stx2e Kylt® E. coli EAST, AIDA, paa
Shiga-toxin forming E. coli (STEC)	Intimin (eae) FimA, FimH	Stx1, Stx2, Stx2e	Kylt® E. coli Stx1, Stx2, eae (Feed & Food safety)

Not all virulence-associated genes of E. coli are of clinical relevance in the different animal species. Some virulence factors are more likely to be found in E. coli strains isolated from swine, others are typical for E. coli isolated from cattle or small ruminants. An overview which virulence factor could be of interest for clinical situations in different livestock animals can be found in table 2.

Table 2: Preferential host for taking sample material for detection of virulence-associated genes of E. coli by using Kylt® Multiplex Real-Time PCR

Multiplex	Art. No		Suckling piglets	weaners	ruminants	feed and food samples
	100 rxn	25 rxn				
Kylt® E. coli Sta, Stb, LT	31706	31707	x	x	x	
Kylt® E. coli F4, F5, F6	31710	31711	x	x		
Kylt® E. coli EAST, AIDA, paa	31714	31715	x			
Kylt® E. coli FimA, FimH, F41	31718	31719	x			
Kylt® E. coli F18, F4, Stx2e	31722	31723		x		
Kylt® E. coli F5, F17, F41	31726	31727			x	
Kylt® E. coli eae, EAST	31730	31731			x	
Kylt® E.coli Stx1, Stx2, eae	31734	31735				x

Suitable sample material:

Typing by Kylt® Multiplex Real-Time PCR systems should be performed with individual isolates of E. coli collected from cultural processes of sample material derived from swine, cattle and small ruminants or feed and food samples.

Contents of the kits:

Kylt® Multiplex Real-Time PCR Kits comprise all reagents and controls for subsequent detection of E. coli virulence-associated genes, including a ready-to-use Reaction Mix for amplification as well as the Positive and Negative Controls.

Advantages

SPEED: Results can be achieved within 2-3 hours. The Real-Time PCR reaction itself takes less than 1.5 hours.

RELIABILITY: The Positive Control monitors the specificity and efficiency of the reagents and of the reaction itself. The Negative Control indicates absence of contaminations.

ACCURACY: The Internal Amplification Control is included in the Reaction Mix in an exact copy number; it is co-amplified in every single reaction to detect possible inhibitory effects of the DNA preparation and to verify true negative results.

The Kylt® Multiplex Real-Time PCR kits can be used on all commercially available Real-Time PCR thermal cyclers detecting the fluorescent dyes FAM, HEX, Cy5 and Texas Red.

Due to identical temperature profiles all Kylt® Multiplex Real-Time PCRs can be combined in one run as well as with other Kylt® Real-Time PCRs.

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