PATHfinder Real-Time PCR kits
for the serotyping of colony isolates of
Shiga toxin-producing E. coli (STEC)

PMB10A-P26 **PMB10A-P103**
PMB10A-P45 **PMB10A-P104**
PMB10A-P111 **PMB10A-P121**
PMB10A-P145 **PMB10A-P157**
PMB10A-H4 **PMB10A-H7**

Introduction

Shiga toxin-producing Escherichia coli (STEC), also known as verocytotoxin-producing E. coli (VTEC), is one of the most common causes of gastrointestinal illness around the world. These foodborne pathogenic bacteria are frequently associated with severe forms of infection including hemorrhagic colitis and hemolytic uraemic syndrome. Most of the STEC foodborne outbreaks with reported known food vehicle were associated with the consumption of food of animal origin (meat, milk and milk products) and with tap water (including well water).

STEC infections are characterized by the production of Shiga toxins (Stx) also referred to as verocytoxins (Vtx); there are 2 major types (Stx1 and Stx2). A third virulence factor, the eae gene, encodes a 90KDa protein, the intimin, which is a typical feature of the pathogenic VTEC strains.

All E. coli can be characterized by their major surface antigens, including somatic (O) antigens and flagellar (H) antigens. STEC O157:H7 is the most frequently reported serogroup in human, however non-O157 VTEC serogroups are also often isolated from human cases, and there are indications that non-O157 serogroups are important causes of severe human infection. Hence, EFSA and other authorities are encouraged to include these serogroups in the monitoring program.

Methods based on Real-Time PCR are the most appropriate approaches to identify serogroups of colony isolates. Five methods were translated into an international standard, ISO/TS 13136:2012, describing the PCR systems for identifying STEC serogroups O157, O111, O26, O103, and O145. Methods for detecting other relevant serogroups were defined by the VTEC European Reference Laboratory.

PATHfinder detection kits provide a simple, reliable, and rapid procedure for detecting the presence of a specific bacterial pathogen. These kits allow the serotyping of STEC isolated on agar plates via colony Real-Time PCR approach. The kit amplifies the target gene and according to ISO 22119 an Internal Amplification Control (IAC) to ensure the reliability of a negative result.

This real-time PCR kit can be run on most real-time PCR instruments like Roche LightCycler 480, Applied Biosystems models, Qiagen Rotor-Gene Q, Bio-Rad CFX models, R-Biopharm RIDA Cycler, Agilent Systems, Hyris bCube, the minimum requirement is the possibility to detect the fluorophores FAM and HEX.

Scientific backing

The kit was developed according to the following documents:

- ISO 22119 "Microbiology of food and animal feeding stuffs — Real-time polymerase chain reaction (PCR) for the detection of foodborne pathogens — General requirements and definitions
- ISO/TS 13136:2012 - Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups
- United States Department of Agriculture (USDA). Primer and Probe Sequences and Reagent Concentrations for non-O157 Shiga Toxin-Producing Escherichia coli (STEC) Real-Time PCR Assay. MLG 5B Appendix 1.01
- Bugarel M, Beutin L, Martin A, Gill A, Fach P. Micro-array for the identification of Shiga toxin-producing Escherichia coli (STEC) seropathotypes associated with Hemorrhagic Colitis and Hemolytic Uremic Syndrome in humans. Int J Food Microbiol. 2010 Sep 1;142(3):318-29.
- Perelle S, Dilasser F, Grout J, Fach P. Detection by 5'-nuclease PCR of Shiga-toxin producing Escherichia coli O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. Mol Cell Probes 2004; 18:185-92.
- VTEC EURL Detection and identification of Verocytotoxin-producing Escherichia coli (VTEC) O104:H4 in food by Real Time PCR

Method and Kit content

Presumptive STEC colonies are isolated from samples on agar plates using a suitable method. After detecting the presence of virulence genes (stx1, stx2 and eae) via colony Real-Time PCR using Generon detection kits, stx positive colonies can be serotyped using these kits and colony PCR method (see product insert for details).

Serotypes are detected by real-time PCR using probes labelled with fluorescence dyes at its 5' and 3' ends (reporter and quencher dye). In parallel, a second PCR system, amplifies and detects an unrelated DNA sequence present in the reagents mix (IAC - Internal Amplification Control) monitoring the quality of the reaction performance.

Each kit contains Polymerase mix with IAC, Primers and probes, Diluent, Positive control, Negative control.

Kit	Gene Serotype	Reporter dye	Quencher	Reference
PMB10A-P26	wzx O26	FAM	BHQ-1	ISO 13136
PMB10A-P45	wzx O45	FAM	BHQ-1	USDA 2012
PMB10A-P103	wzx O103	FAM	BHQ-1	ISO 13136
PMB10A-P104	wzx O104	FAM	BHQ-1	Bugarel et al., 2010
PMB10A-P111	wbdI O111	FAM	BHQ-1	ISO 13136
PMB10A-P121	wzx O121	FAM	BHQ-1	USDA 2012
PMB10A-P145	ihp1 O145	FAM	BHQ-1	ISO 13136
PMB10A-P157	rfbE O157	FAM	BHQ-1	ISO 13136
PMB10A-H4	fliC H4	FAM	BHQ-1	VTEC EURL
PMB10A-H7	fliC H7	FAM	BHQ-1	Perelle et al., 2004
All	IAC-unrelated	HEX	BHQ-1	Proprietary

Exclusivity panel

Duplicate PCR experiments (performed at Generon) loading >2500 genomic copies of the DNA extracted from the following bacteria (65), showed no cross reactivity with the PCR systems:

Aeromonas hydrophila (DSM 30187); Alicyclobacillus acidiphilus (DSM 14558); Alicyclobacillus acidocaldarius subsp. Caldarius (DSM 446); Alicyclobacillus acidoterrestris (DSM 0265P); Alicyclobacillus herbarius (DSM 13609); Bacillus cereus cereulide (DSM 4312); Bacillus cereus (DSM 31); Bacillus subtilis subsp. Spizizenii (DSM 347); Campylobacter coli (DSM 4689); Campylobacter fetus (DSM 5361); Campylobacter jejuni (DSM 4688); Campylobacter laridis (lari) (DSM 11375); Citrobacter freundi (DSM 30039); Citrobacter koseri (ATCC 27028); Clostridium difficile (DSM 1296); Clostridium perfringens (DSM 2943); Clostridium sporogenes (DSM 795); Cronobacter sakazakii (DSM 4485); Edwardsiella tarda (DSM 30052); Enterobacter aerogenes (ATCC 13048); Enterobacter cloacae (DSM 30054); Escherichia coli (DSM 30083); Hafnia alvei (ATCC51873); Janthinobacterium lividum (DSM 1522); Lactococcus lactis subsp. Lactis (DSM 20481); Legionella anisa (DSM 17627); Legionella pneumophila serogroup 1 (DSM 7513); Listeria innocua (DSM 20649); Listeria ivanovi (DSM 20750); Listeria monocytogenes (DSM 20600); Morganella morgani subsp. Morganii (DSM 30164); Proteus mirabilis (DSM 4479); Proteus vulgaris (DSM 13387); Providencia stuartii (DSM 4539); Pseudomonas aeruginosa (DSM 50071); Pseudomonas fragi (DSM 3456); Pseudomonas syringae (DSM 10604); Pseudomonas fluorescens (DSM 50090); Salmonella enterica subsp. Enterica (DSM 17420); Serratia marcescens (DSM 30121); Shewanella putrefacens (DSM 6067); Shigella boydii (DSM 7532); Shigella flexneri (DSM 4782); Shigella sonnei (DSM 5570); Staphilococcus aureus subsp. Aureus (DSM 20231); Staphilococcus caprae (DSM 20608); Staphilococcus epidermidis (DSM 20044); Streptococcus faecalis (ATCC 8043); Vibrio aerogenes (DSM 14438); Vibrio algynoliticus (DSM 2171); Vibrio anguillarum (DSM21597); Vibrio campbellii (DSM19270); Vibrio cholerae (DSM100200; ATCC 14035); Vibrio crassostreae (DSM 17220); Vibrio fischeri (DSM 7151); Vibrio fluvialis (DSM 19283); Vibrio harveyi (DSM19623); Vibrio mediterranei (DSM 13774); Vibrio mimicus (DSM 19130); Vibrio mytili (DSM 19137); Vibrio ordalii (DSM 19621); Vibrio orientalis (DSM 19136); Vibrio parahaemolyticus (DSM 10027; ATCC 17802); Vibrio pelagius (DSM21205); Vibrio splendidus (DSM 19640); Vibrio vulnificus (DSM 10143; ATCC 27562); Yersinia enterocolitica (DSM 27689); Yersinia pseudotuberculosis (DSM 8992).