VIASURE *MULTIPLEX*

E. coli Typing Real Time PCR Detection Kit

Pathogen and product description

Escherichia coli (E. coli) is a gram-negative microorganism that can be an innocuous resident of the gastrointestinal tract, but it also has the pathogenic capacity to cause enteric disease, and extraintestinal diseases, as urinary tract infections (UTIs) and sepsis/meningitis. Pathogenic variants of *E. coli* (pathovars or pathotypes) cause much morbidity and mortality worldwide, due to they have low infectious doses and are transmitted through ubiquitous mediums, including food and water. Of the strains that cause diarrhoeal diseases, six pathotypes are now recognized: Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC), and Diffusely adherent *E. coli* (DAEC).

Enterohaemorrhagic *E. coli* (EHEC) is a subset of Shiga toxin-producing *E. coli* (STEC), also called verotoxin producing *E. coli.* STEC are a diverse group of food-borne pathogens which cause a wide spectrum of human diseases, ranging from mild diarrhoea to severe human diseases, including hemorrhagic colitis (HC) and a lifethreatening complication hemolytic uremic syndrome (HUS). STEC and EHEC strains can be transmitted to humans through person-to-person contact; consumption of raw or undercooked meat, raw milk and other dairy products; ingestion of other food or drinking water contaminated with animal faeces; direct contact with domestic cattle and other ruminants recognised as a major reservoir, and contaminated bathing/ recreational water.

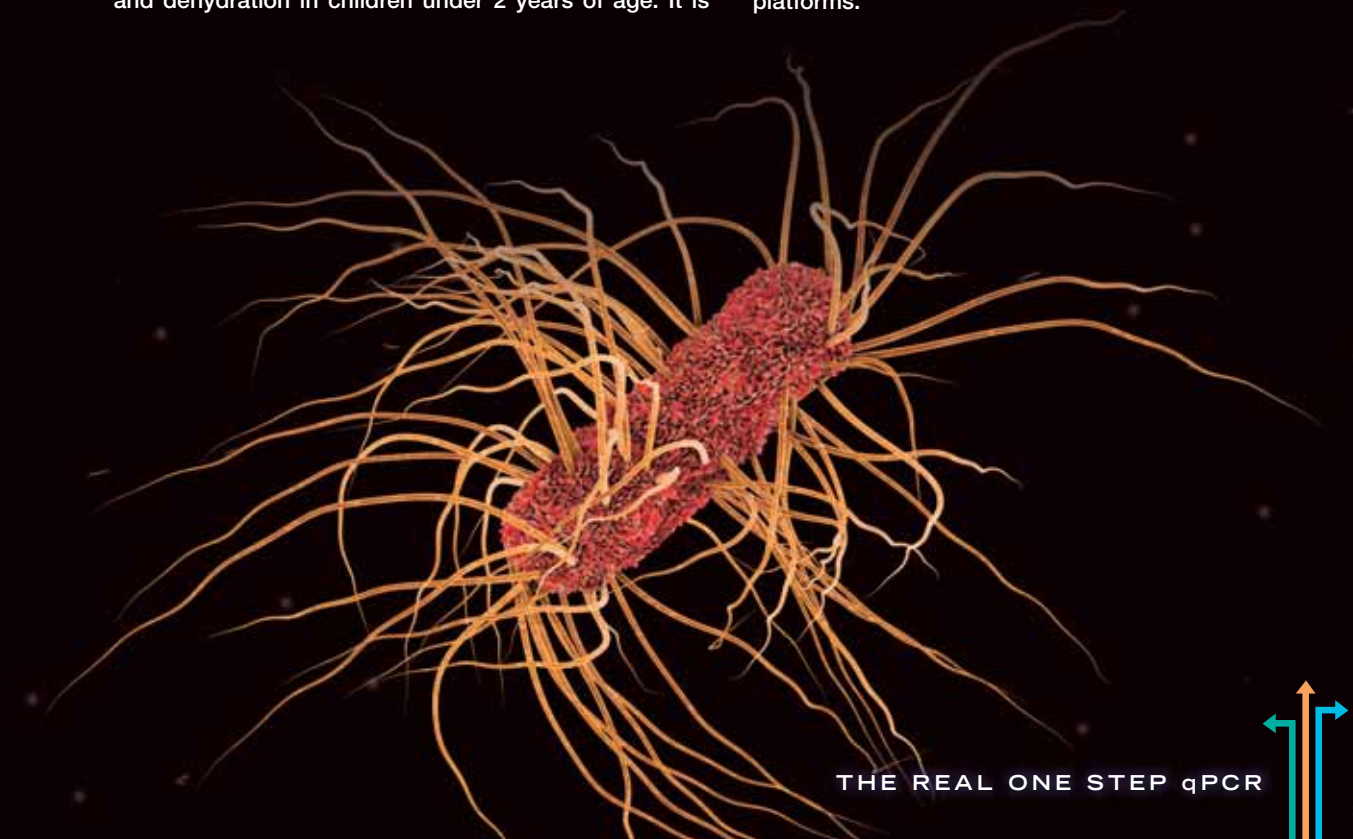
Enteropathogenic *E. coli* (EPEC) also contains eae as EHEC, but without shiga-like toxin. EPEC is an important cause of potentially fatal infant diarrhoea in developing countries that is often accompanied by fever, vomiting, and dehydration in children under 2 years of age. It is transmitted via the fecal-oral route through contaminated surfaces, weaning fluids, and human carriers.

Enterotoxigenic *E. coli* (ETEC) is a major cause of traveller's diarrhoea worldwide and and endemic in most developing countries with significant mortality rates in children. ETEC infections are also transmitted through the fecal-oral route, when a person ingests food or water contaminated.

Enteroinvasive *E. coli* (EIEC) are biochemically, genetically and pathogenically closely related to *Shigella* spp. This infection is characterized by fever, abdominal cramps and diarrhoea containing blood and mucous. Conventional transmission of EIEC and *Shigella* is mediated via the fecal-oral route mainly through contaminated food or water or direct person-to-person spread.

VIASURE *E. coli Typing* Real Time PCR Detection Kit is designed for the diagnosis of EHEC, STEC, EPEC, ETEC and EIEC/*Shigella* in human stool samples. After DNA isolation, the identification of EHEC, STEC, EPEC, ETEC and EIEC/*Shigella* is performed by the amplification of a conserved region of the *stx1, stx2, eae, lt, st1a, st1b* and *ipaH* genes using specific primers and a fluorescent-labeled probe.

VIASURE *E. coli Typing* Real Time PCR Detection Kit is based on the 5´ exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bounded to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of target template. This fluorescence can be measured on Real Time PCR platforms.



Analytical sensitivity

VIASURE *E. coli Typing* Real Time PCR Detection Kit has a detection limit of ≥10 DNA copies per reaction for *stx1/stx2, eae, IpaH, It* and *st* genes (Figures 1, 2, 3, 4, 5 and 6).

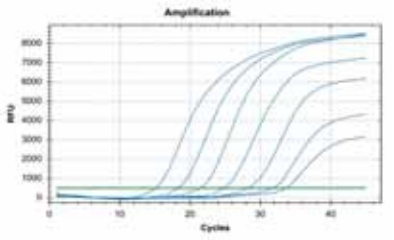


Figure 1. Dilution series of stx1/stx2 genes (10^7–10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (Multiplex reaction mix E. coli EHEC, EPEC & EIEC, channel FAM).

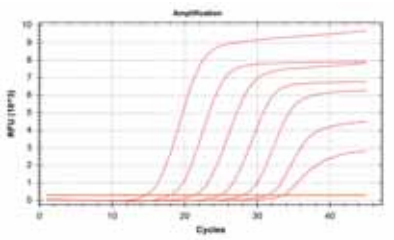


Figure 2. Dilution series of IpaH gene (10^7–10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (Multiplex reaction mix E. coli EHEC, EPEC & EIEC, channel ROX).

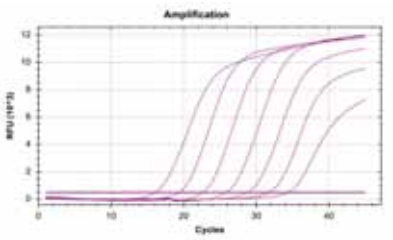


Figure 3. Dilution series of eae gene (10^7–10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (Multiplex reaction mix E. coli EHEC, EPEC & EIEC, channel Cy5).

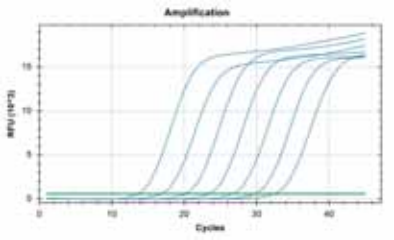


Figure 4. Dilution series of It gene (10^7–10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (Multiplex reaction mix E. coli ETEC + EIEC, channel FAM).

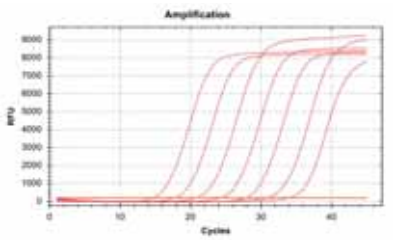


Figure 5. Dilution series of IpaH gene (10^7–10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (Multiplex reaction mix E. coli ETEC + EIEC, channel ROX).

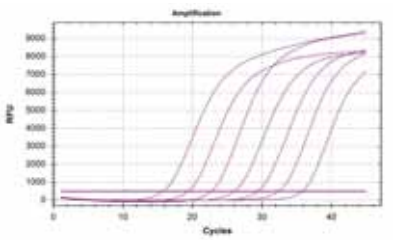


Figure 6. Dilution series of st1a/st1b genes (10^7–10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (Multiplex reaction mix E. coli ETEC + EIEC, channel Cy5).

Components

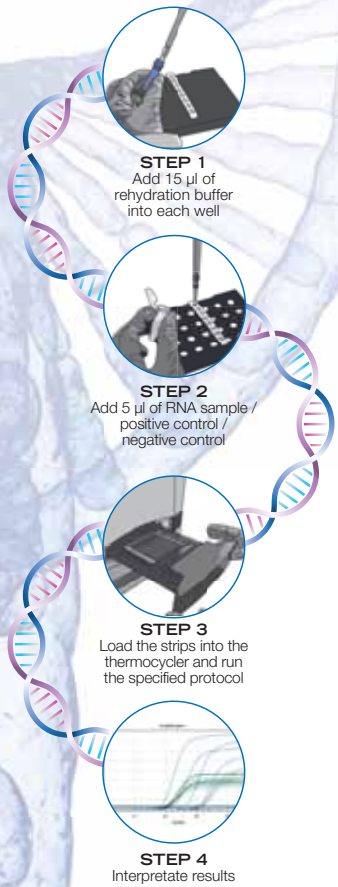
Reagent/Material	Description	Colour	Amount
***E. coli* EHEC + EPEC + EIEC** 8-well strips	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and Internal control in stabilized format	White	3/6 x 8-well strip
***E. coli* ETEC + EIEC** 8-well strips	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and Internal control in stabilized format	White	3/6 x 8-well strip
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1,8 mL
***E. coli Typing* Positive Control**	Non-infectious synthetic lyophilized cDNA	Red	1 vial
Negative Control	Non template control	Violet	1 vial x 1 mL
Water RNAse/DNAse free	RNAse/DNAse free water	White	1 vial x 1 mL
Tear-off 8-cap strips	Optical caps for sealing wells during thermal cycling	Transparent	6/12 x 8-cap strip

Kit References

Reference	Description
VS-ECT106L	VIASURE *E. coli Typing* Real Time PCR Detection Kit 6 x 8-well strips, low profile
VS-ECT106H	VIASURE *E. coli Typing* Real Time PCR Detection Kit 6 x 8-well strips, high profile
VS-ECT112L	VIASURE *E. coli Typing* Real Time PCR Detection Kit 12 x 8-well strips, low profile
VS-ECT112H	VIASURE *E. coli Typing* Real Time PCR Detection Kit 12 x 8-well strips, high profile

Work Flow

One-step rehydration of wells and add your extracted RNA



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