

VIASURE MULTIPLEX

E. coli EHEC + EPEC + EIEC Real Time PCR Detection Kit

Pathogen and product description

E*scherichia 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. Six pathotypes are now recognized: Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteragggregative *E. coli* (EAaggEC), 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.

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. Severe shigellosis complications are often associated with the Shiga toxin-producing serotype *S. dysenteriae* 1 and can range from local intestinal disorders to systemic manifestations. Instead, EIEC might cause an invasive inflammatory colitis, and occasionally dysentery, but in most cases EIEC elicits watery diarrhoea that is indistinguishable from that due to infection by other *E. coli* pathogens. 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* EHEC + EPEC + EIEC Real Time PCR Detection Kit is designed for the diagnosis of EHEC, EPEC and/or EIEC/*Shigella* in human stool samples. After DNA isolation, the identification of EHEC, EPEC and/or EIEC/*Shigella* is performed by the amplification of a conserved region of the *stx1*, *stx2*, *eae* and *lpaH* genes using specific primers and a fluorescent-labeled probe.

VIASURE *E. coli* EHEC + EPEC + EIEC Real Time PCR Detection Kit contains in each well all the components necessary for real time PCR assay (specific primers/ probes, dNTPS, buffer, polymerase) in an stabilized format, as well as an internal control to discard the inhibition of the polymerase activity.



Analytical sensitivity

VIASURE *E. coli* EHEC + EPEC + EIEC Real Time PCR Detection Kit has a detection limit of ≥ 10 DNA copies per reaction for *stx1*, *stx2*, *eae* and *lpaH* genes (Figures 1, 2 and 3).

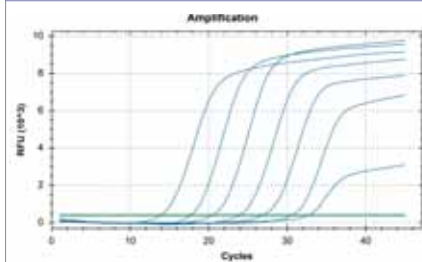


Figure 1. Dilution series of *stx1/stx2* genes (10^7 – 10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (channel FAM).

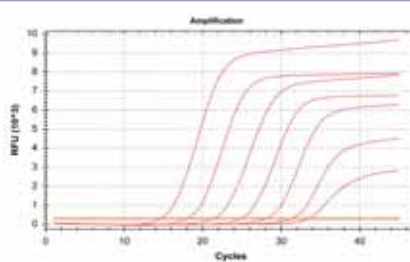


Figure 2. Dilution series of *lpaH* gene (10^7 – 10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (channel ROX).

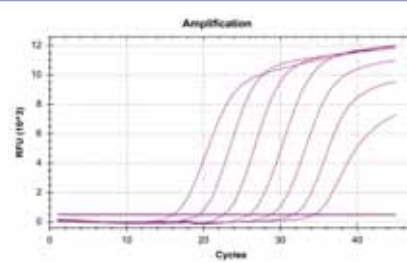


Figure 3. Dilution series of *eae* gene (10^7 – 10^1 copies/rxn) template run on the Bio-Rad CFX96TM Real-Time PCR Detection System (channel Cy5).

Components

Reagent/Material	Description	Colour	Amount
<i>E. coli</i> EHEC + EPEC + EIEC 8-well strips	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and Internal control in stabilized format	White	6/12 x 8-well strip
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1,8 mL
<i>E. coli</i> EHEC + EPEC + EIEC 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

Work Flow

One-step rehydration of wells and add your extracted DNA



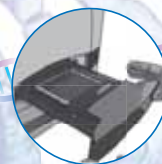
STEP 1

Add 15 μ L of rehydration buffer into each well



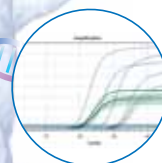
STEP 2

Add 5 μ L of DNA sample / positive control / negative control



STEP 3

Load the strips into the thermocycler and run the specified protocol



STEP 4

Interpretate results

Kit References

Reference	Description
VS-EEE106L	VIASURE <i>E. coli</i> EHEC + EPEC + EIEC Real Time PCR Detection Kit 6 x 8-well strips, low profile
VS-EEE106H	VIASURE <i>E. coli</i> EHEC + EPEC + EIEC Real Time PCR Detection Kit 6 x 8-well strips, high profile
VS-EEE112L	VIASURE <i>E. coli</i> EHEC + EPEC + EIEC Real Time PCR Detection Kit 12 x 8-well strips, low profile
VS-EEE112H	VIASURE <i>E. coli</i> EHEC + EPEC + EIEC Real Time PCR Detection Kit 12 x 8-well strips, high profile
VS-EEE113L	VIASURE <i>E. coli</i> EHEC + EPEC + EIEC Real Time PCR Detection Kit 96-well plate, low profile
VS-EEE113H	VIASURE <i>E. coli</i> EHEC + EPEC + EIEC Real Time PCR Detection Kit 96-well plate, high profile