

# VIASURE MULTIPLEX

## *E. coli* ETEC + EIEC Real Time PCR Detection Kit

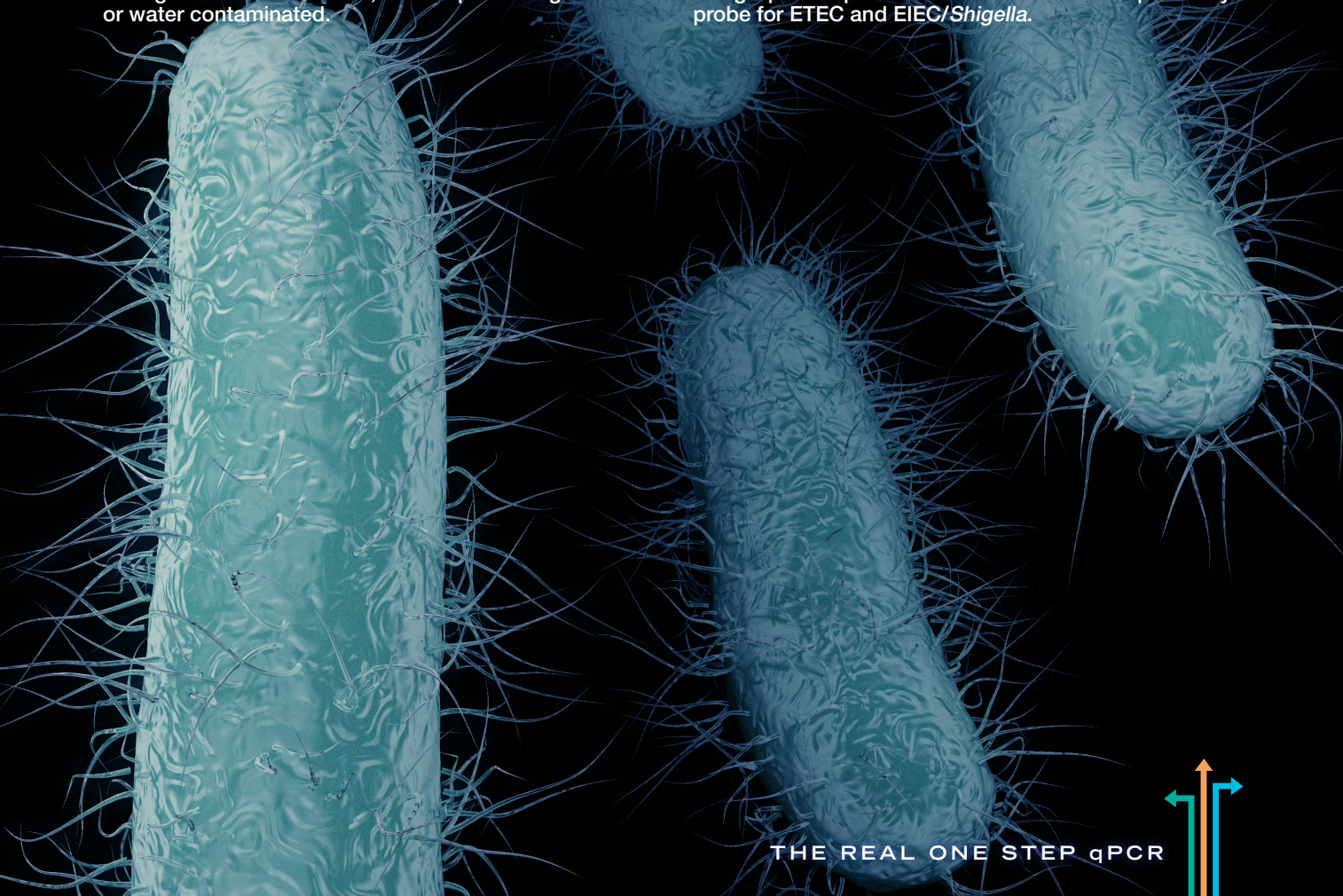
### Pathogen and product description

**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. 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* (EAaggEC), and Diffusely adherent *E. coli* (DAEC). Furthermore, different *E. coli* strains may belong to more than one pathotype group owing to the expression of different virulence factors.

Enterotoxigenic *E. coli* (ETEC) colonizes the surface of the small bowel mucosa and induces watery diarrhoea by the secretion of heat-labile (LT) and/or heat-stable (ST) enterotoxins. These enterotoxins cause inhibition of sodium absorption and stimulation of chloride secretion, which leads to watery diarrhoea. Abdominal cramps, sometimes with nausea and headache, occur and fever is usually absent. ETEC is a major cause of traveller's diarrhoea worldwide and is endemic in most underdeveloped 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.* Both bacteria express the invasion plasmid antigen H (ipaH) gene which is related to invasion. There are four *Shigella* species responsible for human disease (*S. dysenteriae*, *S. flexneri*, *S. sonnei* and *S. boydii*), which cause varying degrees of dysentery. 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* ETEC + EIEC Real Time PCR Detection Kit is designed for the specific identification and differentiation of Enterotoxigenic *E. coli* (ETEC) and Enteroinvasive *E. coli* (EIEC)/*Shigella* in human stool samples from patients with signs and symptoms of gastrointestinal infection. This test is intended to be used as an aid in the diagnosis of ETEC and EIEC/*Shigella* in combination with clinical and epidemiological risk factors. DNA is extracted from stool specimens, multiplied using Real Time amplification and detected using specific primers and a fluorescent reporter dye probe for ETEC and EIEC/*Shigella*.





## Analytical sensitivity

**VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit** has a detection limit of  $\geq 10$  DNA copies per reaction for *It* gene, *IpaH* gene and *st1a/st1b* genes (Figures 1, 2 and 3).

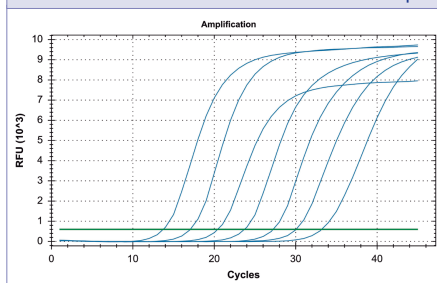


Figure 1. Dilution series of *It* gene ( $10^7$ – $10^1$  copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (channel FAM).

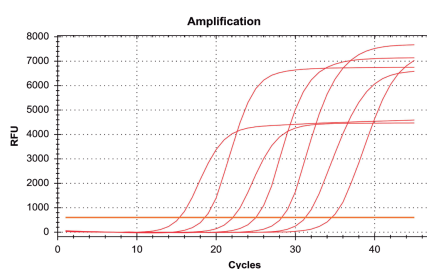


Figure 2. Dilution series of *IpaH* gene ( $10^7$ – $10^1$  copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (channel ROX).

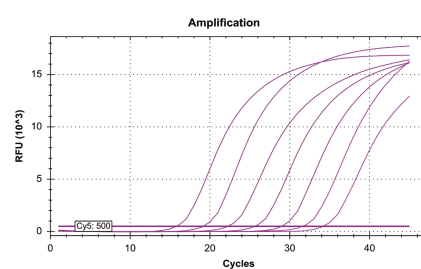


Figure 3. Dilution series of *st1a/st1b* genes ( $10^7$ – $10^1$  copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (channel Cy5).

## Components

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

## Kit References

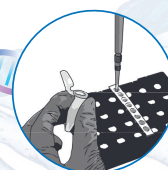
Reference	Description
VS-ESE106L	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 6 x 8-well strips, low profile
VS-ESE106H	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 6 x 8-well strips, high profile
VS-ESE112L	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 12 x 8-well strips, low profile
VS-ESE112H	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 12 x 8-well strips, high profile
VS-ESE113L	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 96-well plate, low profile
VS-ESE113H	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 96-well plate, high profile
VS-ESE136	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 9 x 4-well strips, Rotor Gene®
VS-ESE172	VIASURE E. coli ETEC + EIEC Real Time PCR Detection Kit 18 x 4-well strips, Rotor Gene®

## Work Flow

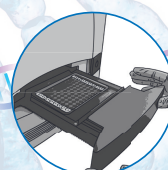
One-step rehydration of wells and add your extracted DNA



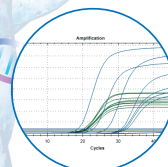
**STEP 1**  
Add 15 µl of rehydration buffer into each well



**STEP 2**  
Add 5 µl of DNA sample / positive control / negative control



**STEP 3**  
Load the strips into the thermocycler and run the specified protocol



**STEP 4**  
Interpretate results



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