CE

PCRFast® Human Diagnostics

VTEC stx1 / 2

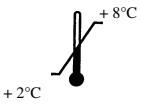
Real - time PCR Kit

Ready - to - use 0.2 ml PCR tubes

PSTE0060S

REF

PSTE0060S



IVD

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PCRFast® VTEC stx1 / 2

1. Intended use

PCRFast® VTEC stx1 / 2 is intended for the qualitative detection of verotoxin producing *Escherichia coli*, VTEC (synonym “shiga toxin producing *E. coli*, STEC”) in stool samples.

For *in vitro* diagnostics only.

2. Principle of the test

PCRFast® VTEC stx1 / 2 is an easy to use molecular biological test (real - time PCR) for the detection of VTEC stx1 / 2 in faeces (detection of the genes for stx1 and stx2). The analytical procedure described here complies with the international standards (e. g. ISO / DIN 20838) for PCR analysis.
The kit contains ready - to - use PCR reaction tubes with lyophilized reagents for reconstitution with MasterMix. MasterMix is dispensed into the PCR reaction tube, and the extracted sample DNA is added. The target sequence is amplified and detection is carried out in real - time by means of specific probes.

3. Kit contents

Table 1 shows the components that are included in the kit:

Table 1: Kit contents

Tube	Content
11 x strips with 8 PCR tubes each (0.2 ml, colourless)	Specific primers, probes and internal amplification control *lyophilized; for samples and negative controls*
1 x strip with 8 PCR tubes (0.2 ml, red mark)	Specific primers, probes, internal amplification control and homologous VTEC stx1 / 2 DNA *Lyophilized; for positive controls*

4. Required instruments and reagents

4.1 Instruments and accessories

- PCR thermal cycler
Applied Biosystems (ABI) 7500, 7700, 7900
Stratagene Mx3005P, Mx3000P
- Microliter pipettes with sterile filter tips
- Tabletop centrifuge with rotor for 0.2 ml PCR tubes, minimum 1,000 revolutions per minute (rpm)

4.2 Reagents

- MasterMix for reconstitution (for recommendations, see Table 2)
- 0.1 x TE buffer (e. g. 1 x TE buffer from Sigma, 93283; diluted 1 : 10 with water, PCR grade)
- Water, PCR grade (e. g. Sigma, W1754)

Table 2: MasterMix recommendations

Cycler	MasterMix
Stratagene Mx3000P, Mx3005P	Brilliant® II QPCR MasterMix Kit, Stratagene
ABI 7500, 7700, 7900	Rotor - Gene Probe PCR Kit, Qiagen

5. Storage of reagents

Store the kit / the vials at 2 - 8 °C.

6. Stool extraction

For the extraction follow the manufacturer's instructions (e. g. NucliSENS® easyMAG™, NucliSENS® miniMAG™ [bioMérieux], QIAamp® DNA Stool Mini [Qiagen]).

Note: Stool samples may inhibit PCR. In this case dilute the extract.

7. PCR setup

1) Remove strips from plastic bag and clip off the required number of colourless PCR tubes
2) For the positive control, clip off one PCR tube marked in red
3) Place the remaining strips / tubes and the desiccant back into the bag, seal properly and store at 2 - 8 °C
4) **Dispense 12.5 µl of 2 x concentrated MasterMix** into each 0.2 ml PCR tube
5) Add **12.5 µl of the extracted sample** (colourless PCR tubes)
6) For the negative control, add **12.5 µl of 0.1 x TE buffer** instead of sample (colourless PCR tube)
7) For the second control, add **12.5 µl of the negative extraction control** (colourless PCR tube)
8) For the positive control, add **12.5 µl of 0.1 x TE buffer** (PCR tube marked in red)

Seal tubes / plate properly and centrifuge.

Note: For controls, see Section 16.2

8. Instrument settings

Reporter: FAM (520 nm) and HEX (555 nm)
Quencher: no fluorescence

Table 3: Temperature profile

Segment	Hold time	Temperature	Cycles
1. Activation *Taq* - Polymerase	10 min	95 °C	1 x
2. Denaturation	15 sec	95 °C	45 x
3. Annealing	30 sec	60 °C	
4. Extension	30 sec	72 °C	

9. Analysis

Evaluation matrices are shown in Tables 4 and 5.

Table 4: Controls (**+**: amplification; **-**: no amplification)

Control	Amplification VTEC stx1 / 2 (FAM 520 nm)	Internal amplification control (HEX 555 nm)	Result
Negative control	-	+	Reaction solution not contaminated
Positive control	+	+	Reaction solution functional
Extraction control	-	+	Extraction not contaminated

Table 5: Samples (**+**: amplification; **-**: no amplification)

Amplification VTEC stx1 / 2 (FAM 520 nm)	Internal amplification control (HEX 555 nm)	Result
-	+	Sample negative
+	+ / -	Sample positive
-	-	Inhibition*

* dilute extracted sample and repeat PCR

10. Amplification curves

The following figures show exemplary amplification curves from the Stratagene Mx3005P instrument.

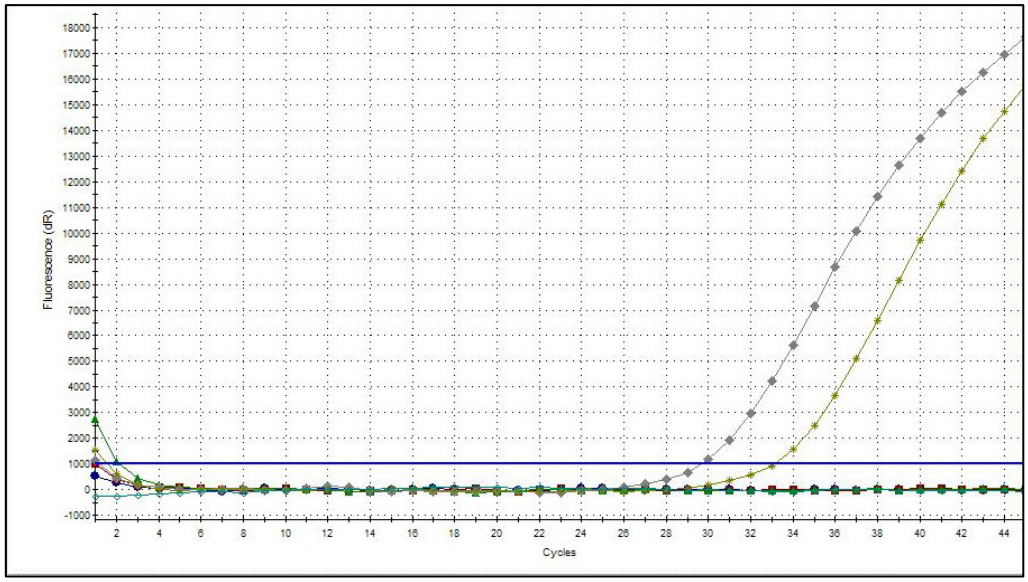


Figure 1: Amplification curves in the FAM channel (FAM filter set): samples

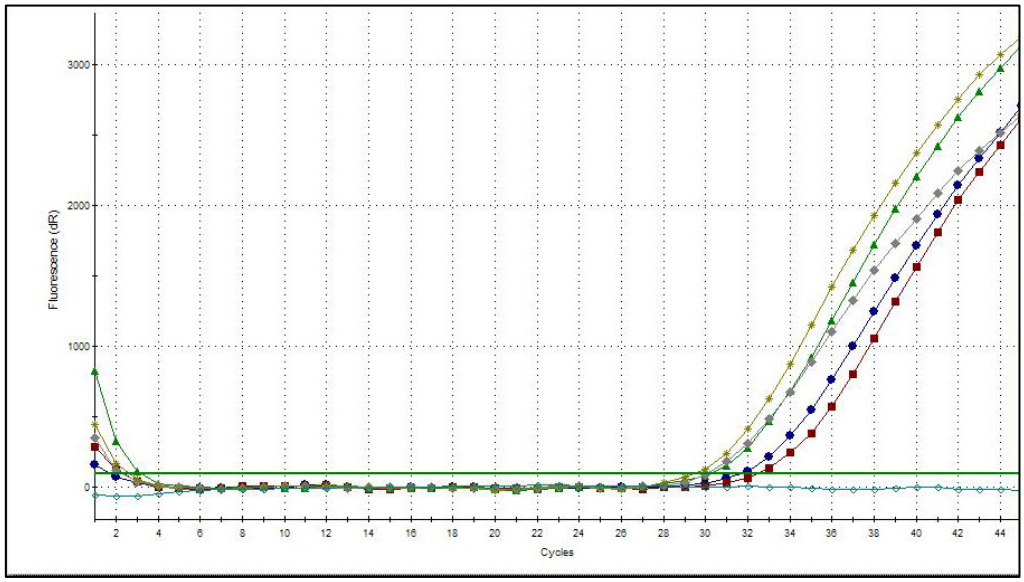


Figure 2: Amplification curves in the HEX channel (HEX filter set): internal transcription / amplification control

11. Performance characteristics

11.1 Diagnostic sensitivity

The diagnostic sensitivity is defined as the probability of scoring positive samples in the presence of the analyte. In the present assay it is > 98 %.

11.2 Diagnostic specificity

The diagnostic specificity is defined as the probability of scoring negative samples in the absence of the analyte. In the present assay it is > 98 %.

11.3 Analytical sensitivity

The limit of detection is < 10 copies per reaction. This equals < 1 x 10^3 VTEC stx1 / 2 per gram of stool.

11.4 Analytical specificity

PCRFast® VTEC stx1 / 2 is specific to VTEC stx1 / 2. The following serotypes and species have been tested for cross - reactivity with at least 2,500 copies each (excerpt):

Table 6: Specificity (**+**: amplification; **-**: no amplification)

Species		Species		Species	
***Escherichia coli* VTEC stx1**	**+**	***Escherichia coli* VTEC stx2**	**+**	*Escherichia coli*	-
Yersinia enterocolitica	-	*Legionella pneumophilia*	-	*Campylobacter jejuni*	-
Staphylococcus aureus	-	*Bacillus cereus*	-	*Salmonella* Abony	-
Clostridium perfringens	-	*Listeria monocytogenes*	-	*Legionella erythra*	-

12. Limitation of procedure

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology, molecular biology as well as serological data.

13. Precautions

- This assay was produced and put on the market according to the IVD guidelines of 98 / 79 / EC. All test procedures, information, precautions and warnings given in this manual must therefore be strictly followed.
- Only for *in vitro* diagnostics.
- Do not use after the expiry date stated on the label.
- All common lab precautions should be taken when performing the test and all PCR steps should be performed in accordance with the CEN / ISO recommendations.
- To avoid carryover contamination, wear protective gloves and use filter tips during the entire test procedure.
- Perform sample preparation, PCR setup and detection in separate rooms.
- All materials possibly containing VTEC stx1 / 2 should be autoclaved (20 min at 121 - 123 °C).

14. Disposal

Dispose of used PCR reaction tubes in the domestic waste.

15. Contact

If you have any questions on how to conduct this test, on sample preparation or on PCR analysis in general, please contact the Competence Centre for PCR Analysis of ifp, Institut für Produktqualität.

info@produktqualitaet.com
Tel. +49 (0) 30 / 76 68 60 - 0
Fax +49 (0) 30 / 76 68 60 - 50

ifp
Institut für Produktqualität

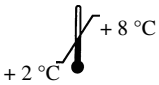
For further information on PCRFast® please visit www.produktqualitaet.com.

16. Appendix

16.1 Symbols key

Manufacturer

In vitro diagnostics



Store at

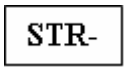
Lot number

Expiry date

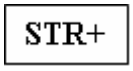
Order number

Number of tests

Please note the instructions



Strips (colourless) with primers, probes and internal amplification control



Strip (marked in red) with primers, probes, internal amplification control and homologous DNA

16.2 Controls

Obligatory and optional controls are listed in Tables 7 and 8.

Table 7: Obligatory controls

Control	Description	Aim	Recommendation
Negative control (brown cap)	0.1 x TE buffer	Testing the reaction solution for contamination	Include in every PCR run
Positive control (red cap)	0.1 x TE buffer	Testing the PCR reaction for functionality	Include in every PCR run
Negative extraction control (brown cap)	Entire extraction without sample material	Testing the extraction reagents for contamination	Test each new extraction series

Table 8: Optional controls

Control	Description	Aim	Recommendation
Negative process control (brown cap)	Sample without target sequence	Negative result (replaces the negative extraction control)	Optional
Positive process control (brown cap)	Sample with target sequence, e. g. reference sample / spiked sample	Positive result	Optional

16.3 Bibliography

Arnheim N., Erlich H.
"Polymerase Chain Reaction Strategy", Annual Review of Biochemistry (1992), **61 XIV** : 131 - 156.

Fürst S., Scheef J., Bielaszewska M. et al.
„Identification and characterisation of Escherichia coli strains of O157 and non-O157 serogroups containing three distinct Shiga toxin genes" J Med Microbiol. 2000 Apr;**49**(4):383-6.

Mackay I.M.
"Real - time PCR in the microbiology laboratory" Clin. Microbiol. Infect. (2004) **10**, 190 - 212.

Schmidt H., Geitz C., Tarr P.I., Frosch M., Karch H.
„Non-O157:H7 Pathogenic Shiga Toxin–Producing *Escherichia coli:* Phenotypic and Genetic Profiling of Virulence Traits and Evidence for Clonality" J Infect Dis. 1999 Jan;**179**(1):115-23.