Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

MAST ISOPLEX® *VTEC* kit

DNA/LYO3 20 tests
For use with the real-time thermal cyclers with FAM, TAMRA and CY5 detection capacity.

Intended Purpose
MAST ISOPLEX® *VTEC* kit is optimised for typing of verotoxigenic *E. coli* (VTEC) from human stool samples with loop-mediated isothermal amplification (LAMP) using MAST ISOPLEX® *Probe* technology. The kit contains reagents for detection of virulence-associated genes of *E.coli*: VTEC variants 1 and 2 (VT1 and VT2). VT1 and VT2 genes can be detected simultaneously in a triplex assay format with an inhibition control DNA (IC DNA).

Components of the kit
1. VTEC LAMP Pellets (PEL1) x 2 pellets. Red Cap Tubes.
2. VTEC Primer and Probe mix with Inhibition Control DNA (PP1) x 2 tubes. White cap tubes.
3. Positive Control DNA (VT1VT2) - Green cap tube.
4. Reconstitution Buffer (RB) x 1 tube. Yellow Cap Tube.
5. Water, molecular grade (WTR) x 1 tube. Black Cap Tube.

Storage and Shelf life
Store the unopened kit at 2°C to 8°C away from direct sunlight until the expiry date shown on the pack label. Once reconstituted, all reagents should be aliquoted and stored at minus 20°C until the expiry date shown on the tube label. Store all other components at 2°C to 8°C away from direct sunlight.

Warnings and Precautions
Precautions should be taken to prevent contamination of reagents in the kit and samples. The kit is designed to be used by trained laboratory personnel only.
Reaction tubes should be kept closed at all times following addition of reagents and discarded without opening following use, according to local health and safety guidelines. To avoid any contamination with the amplified product, never open a vial after amplification. Do not vortex reaction tubes. Ensure all reaction tubes are not scratched or cracked prior to use.

Materials required but not provided
1. DNA extracted from human stool sample to be provided by the user.
2. To ensure the best assay sensitivity it is recommended to use DNA extracts from overnight cultures.
3. DNA from specimen sample should be obtained according to standard laboratory procedures.
4. Standard DNase free supplies such as assay reaction tubes, pipettes and pipette tips.
5. An instrument capable of performing isothermal incubation of reaction tubes at the desired temperature such as the Applied Biosystems (ABI) 7500 FAST REAL-TIME PCR system, equivalent in-house thermocycler. The equipment should have a fluorescent reader with FAM, TAMRA and CY5 detection channels for recognition of amplification products.

Preparation of reagents
Reconstitution of lyophilized components:
Spin tube briefly in a microcentrifuge and ensure the lyophilized reagents pellets are at the bottom of the tube.
Reconstitute reagents as described in Table 1 below.
Mix gently by pipetting up and down several times.
Each reagent tube is sufficient for 10 reactions.

Table 1: Reconstitution of lyophilized components

Component	Diluent	Volume
PEL1	RB	20µl
PP1	H_2O	10µl
VT1VT2 DNA	H_2O	20µl

VTEC assay set-up
Each reconstituted PEL1 can be used for ten assay reactions (10 µl per assay).
Set up the reaction as described in table 2.

Table 2: VTEC assay set-up

VTEC assay set-up	Per sample
PEL1 reconstituted in RB	2µl
Reconstituted PP1	1µl
DNA sample or VT1VT2 DNA (positive control)	1-7µl of DNA sample or 1µl of VT1VT2 PCDNA
WTR	0-6µl
Total reaction volume	10µl

For a negative control, prepare a no-template control (NTC) by replacing DNA sample with molecular grade water (WTR).

Programming Information for selected real-time thermal cycler

Dye selection:
TAMRA for detection of IC
CY5 for detection of VT1
FAM for detection of VT2
Temperature: 63°C
Sample volume: 10µl
ROX (if available): unselected
Assay cut off time: 40 minutes.

Data analysis:
Baseline: 3 to 5 cycles
Threshold set up: above the plot generated by the negative control.

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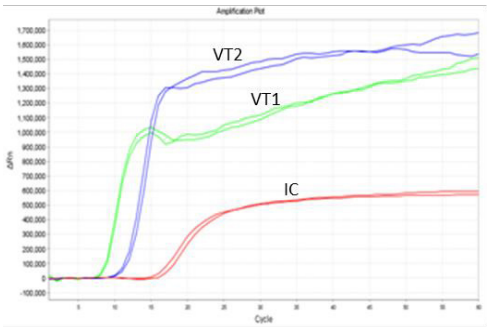
Interpretation of results

Thermocycler results: A positive result is indicated by presence of an amplification curve and a negative result is indicated by fluorescence without amplification within the reaction time as shown on an ABI 7500 FAST REAL-TIME PCR system.

Figure 1: Example of amplification plots for a triplex assay

A – *E.coli* VT1 and VT2 Positive Control



B – No template control

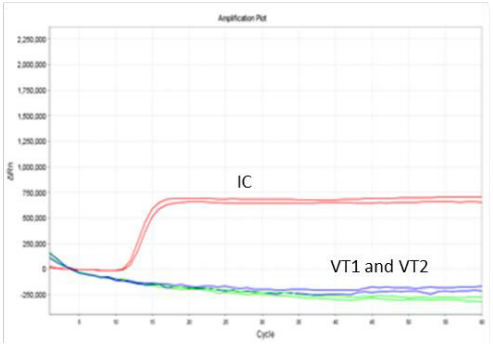


Table 3: Criteria for valid interpretation of VTEC multiplex assay with an inhibition control (IC)

Controls	Target gene (VT1 CY5, VT2 FAM)	IC TAMRA	Interpretation
Negative control	-	+	Valid
Positive control	+	+	Valid
Sample	-	+	Negative for the target gene
Sample	-	-	Invalid
Sample	+	+/- *	Positive for the target gene

* IC TAMRA can occasionally fail to amplify if target DNA is present in extremely high quantities, out-competing IC TAMRA primers and probes for reagents.

Quality control

It is recommended that quality control on the MAST ISOPLEX® *VTEC* kit is performed using the VTEC pellet (PEL1), Positive Control DNA (VT1VT2), the Probes, Primers and IC DNA mix (PP1), pellet reconstitution buffer (RB) and the molecular grade water (WTR) supplied in the kit per test run. These tests will ensure the reagents perform as specified and no contamination of kit reagents has occurred. If control reactions give incorrect results, check for signs of deterioration or contamination of kit reagents and perform a retest. If retest also fails, contact Mast technical support department for advice.

Limitations of use

These products are for use in the amplification of DNA samples using standard DNA extraction methods. Quality Control will determine if kit reagents and controls are functional and free of contamination but cannot determine potential issues the user may experience with samples for DNA extraction. Sample extraction is the sole responsibility of the end user. Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

Specificity/Sensitivity/Limit of Detection (LOD)

Data obtained from DNA extracts from VTEC presumptive faecal sample and VTEC negative culture (n=23).

Sensitivity – 90%

Specificity – 100%

LOD (genome copy number): VT1: 29
VT2: 24

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