# MAST ISOPLEX® *VTEC & MAST ISOPLEX® E.coli O157*
# Frequently asked Questions and Answers

## What is LAMP?

Loop-mediated isothermal amplification (LAMP), a nucleic acid amplification method developed in 2000, combines speed, simplicity, and high specificity. LAMP assays are performed under isothermal conditions using a strand displacement reaction, which employs a DNA polymerase and a set of specially designed primers that recognize distinct sequences on the target DNA.

## What are the stages of the LAMP reaction?

The amplification cycle comprises two principal stages:

1. Copying of the target gene of interest using the specially designed gene primers to form the characteristic dumbbell structure which is the starting point for the LAMP reaction. The formation of the dumbbells is a continuous process, working simultaneously with the amplification reaction.

2. Isothermal amplification builds upon the dumbbell starting unit to form long strands of alternately

inverted repeats of the target molecular sequence on the same strand.

## What is the difference between LAMP and PCR?

The isothermal reaction conditions of a LAMP assay allow for the continuous amplification of target genes, rather than being restricted to short periods of amplification as seen in polymerase chain reaction (PCR) cycling, which relies upon thermal cycling to achieve DNA strand separation, primer annealing and finally target extension. The continuous amplification of LAMP allows for the generation of much larger quantities of nucleic acids in a much shorter period of time. Production of huge quantities of nucleic acids have been reported within 15-60 minutes depending on the target and primer set.

## What is the scope of MAST ISOPLEX®VTEC & MAST ISOPLEX®E.coli O157 kits?

**MAST** ISOPLEX® *VTEC* & **MAST** ISOPLEX®*E.coli O157*: Lyophilised reaction pellets, probes and positive control DNA provided with the kit give the end-user all the constituents necessary for these assays. To run an assay, the end-user simply has to resuspend a lyophilised pellet in reconstitution buffer and water (also supplied with the kit) and add the probes at recommended levels alongside extracted sample DNA, Positive control DNA or water (if running a no-template control test). Each LAMP reaction is incubated at a temperature of 63$^{o}$C for a recommended time.

MAST ISOPLEX®*VTEC* kit is optimised for typing of 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).

MAST ISOPLEX®*E.coli O157*. The kit contains reagents for detection of Perosamine synthetase (RfbE) expressed by *E.coli O157*. *E.coli O157* genes can be detected simultaneously in a duplex assay format with an inhibition control DNA (IC DNA).

## How does the probe technology work?

MAST ISOPLEX ®LAMP assays rely on 6 specific primers that recognise 8 locations within a target DNA sequence. MAST ISOPLEX®Probe technology consists of single gene-specific oligonucleotides labelled with a fluorophore of choice. These oligonucleotides are incorporated into LAMP primer sets against targets of interest and enable accurate, real-time detection and amplification of target DNA. Single stranded primer with a fluorophore binds to DNA that has been displaced by DNA Polymerase which results in extended primer fluorescence due to primer elongation into a double stranded product. **See Patent WO2015/063498 for more in-depth analysis of probe function.**

## How specific and sensitive are the kits?

Validations were performed in these kits using DNA extracts from VTEC and *E. coli O157* presumptive faecal samples as well as negative cultures, which yielded excellent sensitivity and specificity.
A total of 23 samples were tested for VT1 and VT2 during the evaluation of MAST ISOPLEX®*VTEC* with the results yielding a sensitivity of 90% and a specificity of 100%. A total of 23 samples were tested for *E.coli O157* during the evaluation of MAST ISOPLEX®*E.coli O157* with the results yielding a sensitivity of 100% and specificity of 100%. A limit of detection (LOD) of 29 for VT1 and 24 for VT2 were determined for MAST ISOPLEX®*VTEC* kits. A LOD of 1166 for *E.coli O157* was determined for MAST ISOPLEX®*E.coli O157* kits.

## Does the MAST ISOPLEX® VTEC and MAST ISOPLEX® E.coli O157 require primer design?

No a VTEC or *E.coli O157* Primer and Probe mix with Inhibition Control DNA is contained within each kit. Primer design is not required, simply provide extracted target DNA.

## What equipment is needed to amplify MAST ISOPLEX®VTEC and MAST ISOPLEX® *E.coli* O157 reagents via LAMP assay?

An instrument capable of isothermal incubation of reaction tubes at the desired temperature (60-65°C) such as the Applied Biosystems (ABI) 7500 FAST REAL-TIME PCR system or equivalent in-house thermocycler. The equipment should have a fluorescent reader with FAM, CY5 or TAMRA detection channel for MAST ISOPLEX® *VTEC* and FAM or TAMRA detection channel for MAST ISOPLEX® *E.coli O157* for recognition of amplification products.

## What is the shelf life? How should I store these kits?

MAST ISOPLEX®*VTEC* and MAST ISOPLEX®*E.coli O157* reagents have a shelf life of one year and it is recommended that customers and end-users of the MAST ISOPLEX®*VTEC* & MAST ISOPLEX®*E.coli O157* kits store them between 2-8ºC.

## What DNA preparation method is needed?

Although LAMP reagents are traditionally less susceptible to inhibitors compared to standard PCR components, we recommend using DNA extracts from overnight cultures to obtain suitable specificity and sensitivity data. MAST ISOPLEX®*VTEC* and MAST ISOPLEX®*E.coli O157* require extracted DNA for effective typing of *E. coli* toxins from human stool samples. Performing LAMP assays directly from faecal extracts can work but is very likely to compromise sensitivity. DNA should be extracted and stored according to standard laboratory procedures. Sample extraction is the sole responsibility of the end user.

## What does a positive result look like?

A positive result on a thermocycler is indicated by presence of an amplification curve and a negative result is indicated by fluorescence without amplification within the reaction time as shown typically as an 'S' shaped sigmoid curve on an ABI 7500 FAST REAL-TIME PCR system, indicating the increase in fluorescence observed on detection of positive sample DNA.

## How fast should I expect a result?

Positive results can be obtained with MAST ISOPLEX®*VTEC* and MAST ISOPLEX®*E.coli O157* reagents between 5 and 40 minutes.

## What is the optimal temperature for running LAMP reactions?

MAST recommends using MAST ISOPLEX®*VTEC* and MAST ISOPLEX®*E.coli O157* reagents in LAMP reactions at 63°C.

## What is an Inhibition Control and how does it differ from a positive control?

Inhibitors can be found in various specimen matrices, and these substances can interfere with the LAMP reaction by interacting directly with DNA and blocking the activity of the polymerase or other assay reagent, thereby preventing target amplification. Inhibition control primers and DNA are incorporated into assay components during kit manufacturing and are used to reduce the likelihood of false-negative results due to inhibition i.e. Failure of these assays usually indicate that kit components are inhibited or degraded. Unlike Inhibition control tests, positive control experiments can tell if a sample assay is functional but, if negative, cannot determine if the fault for experimental failure lies with kit reagents or the quality of sample used. Positive control DNA and/or primers are also typically added externally to positive control tests while inhibition control DNA and primers are usually integral to the reagents being analysed.

## If the Inhibition Control does not amplify, does that always mean the sample is not valid?

In MAST ISOPLEX®*VTEC* and MAST ISOPLEX®*E.coli O157* tests, 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. Please refer below for criteria on valid interpretation

MAST ISOPLEX® *VTEC*

- If the negative control is negative and the Inhibition control is positive this is a valid result
- If the positive control is positive and the inhibition control is positive, this is a valid result
- If the sample is negative and the inhibition control is positive the result is negative for the target gene
- If the sample Is negative and the inhibition control is negative, the result is invalid
- If the sample is positive and the inhibition control is positive, the result is positive for the target gene

MAST ISOPLEX® *E.coli O157*

- If the negative control is negative and the Inhibition control is positive this is a valid result
- If the positive control is positive and the inhibition control is positive, this is a valid result
- If the sample is negative and the inhibition control is positive the result is negative for the target gene
- If the sample Is negative and the inhibition control is negative, the result is invalid
- If the sample is positive and the inhibition control is positive, the result is positive for the target gene