

# EVALUATION OF A PORTABLE AMPLIFICATION PLATFORM FOR LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) AND AFRICAN SWINE FEVER (ASFV)



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LAMP, Portable amplification platform, FMDV, ASFV

## Introduction

Rapid, sensitive, inexpensive, and easy to use detection methods and equipment are important for fast detection of highly infectious diseases such as Foot-and-Mouth Disease (FMD) and African Swine Fever (ASF) whose outbreaks can result in severe economic losses. FMD and ASF are caused by Foot-and-Mouth Disease virus (FMDV) and African Swine Fever virus (ASFV) respectively, which infect pigs as well as wildlife and other domestic animals. Real-time PCR has been shown to be a powerful and sensitive tool for routine diagnostic detection of FMDV and ASFV. However, it relies on precision thermocycling and requires instrumentation which can be fragile and expensive. LAMP provides an alternative to PCR-based detection assays due to its simple operation, equal sensitivity and rapid target detection.



**Figure 1:** The GENIE detection system allows real-time LAMP and is designed for use with LAMP assays. It has two independent heating blocks that can each process 1-8 samples and can perform temperature gradient.

This study is an initial evaluation of the GENIE I machine and the accompanying enzymology from Optigene using isothermal amplification methods (LAMP) previously developed for the detection of FMDV (1) and ASFV (2).

## Materials & methods

Decimal dilutions of a ~10% epithelial suspension of ASFV isolate Malta78 and FMDV virus isolate O/UKG 35/2001 taken from the archival material stored at the reference laboratory Institute for Animal Health, Pirbright were prepared in suspensions of uninfected pig spleen homogenates and bovine epithelium, respectively.

Nucleic acid template for each sample in the dilution series of ASFV was extracted using the QIAamp viral RNA mini kit (QIAGEN), whereas FMDV samples were extracted using the RNeasy mini kit (QIAGEN) according to manufactures protocol. Total nucleic was stored in aliquots at -70°C until required for subsequent testing. Uninfected pig spleen homogenates and bovine epithelium were used as a negative control for each dilution series.

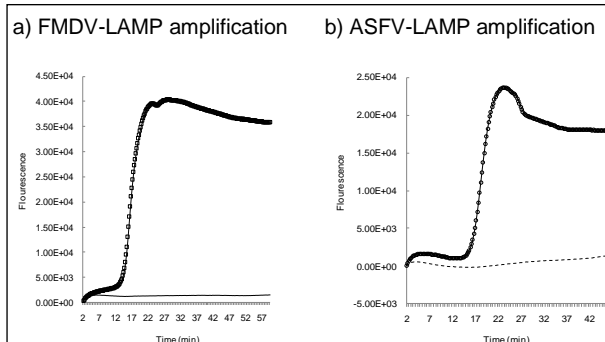
The LAMP assays specific for ASFV topoisomerase II (2) and 3D RNA polymerase-encoding region of the FMDV genome (1) as previously described were carried out on all extractions and run in parallel on the GENIE1 (Optigene) as well as Mx3005P (Stratagene). Additionally the samples were tested using the enzyme mastermix (Isothermal Mastermix) provided by Optigene in comparison to the developed 'in-house assay' using Bst polymerase (NEB).

## Results

Initial experiments of LAMP using neat suspensions of FMDV and ASFV performed well on the GENIE 1 with O/UKG 35/2001 being detected at approximately 13min and Malta78 is detected at approximately 15min. (Figure 2)

The decimal dilutions of O/UKG 35/2001 and Malta78 were tested by comparing the enzyme mastermix (Isothermal Mastermix) provided by Optigene with the 'in-house' LAMP assays and results showed that the Optigene enzyme mastermix detected virus considerably earlier than the 'in-house assays' with a difference of up to 10-15min, although analytical sensitivity was equivalent between the two reagents.

Additionally, a decimal dilution series of O/UKG 35/2001 and Malta78 were run on GENIE 1 (Optigene) and Mx3005P (Stratagene) in comparison using the 'in-house' LAMP assays. Results indicate that both machines performed equally well in running LAMP and gave similar analytical sensitivity results between the two platforms for the detection of FMDV and ASFV.



**Figure 2:** Detection of FMDV and ASFV isolate using real-time LAMP on GENIE 1: (□) OUKG35/2001 (—) Uninfected bovine epithelium (○) Malta78 (---) Uninfected pig spleen

## Discussion & conclusions

Both, the FMDV and ASFV LAMP assay transferred easily from a lab-based PCR machine on to the GENIE 1 (Optigene). The LAMP reaction run for both viruses is a single tube assay, running at a constant temperature for less than an hour, which makes it an easy to run test for diagnosis with rapid results that are easy to interpret. The use of the enzymology provided by Optigene enables accelerated ASFV and FMDV detection and can produce test results within shorter time-scales. Therefore, this modified LAMP assay should prove to be a valuable tool in the laboratory diagnosis of ASFV and FMDV. Furthermore, the Genie 1 (Optigene) performs well in comparison to the Mx3005P (Stratagene). Considering its comparatively light weight, size, battery power and user friendliness the GENIE 1 (Optigene) would be highly suited for use in non-specialised or mobile laboratories for near-farm diagnosis.

In conclusion, the GENIE 1 is a reliable device for rapid FMDV or ASFV detection using LAMP, which in conjunction with Optigene enzymology can potentially accelerate and improve the efficiency of FMDV or ASFV diagnosis for future outbreaks of these economically important viruses.

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