

Development of an RT- LAMP assay to detect the Classical Rabies



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Background:

- There is no terrestrial rabies in the UK but there are related viruses in bats
- Rapid antemortem diagnosis ensures appropriate patient management
- There are 55,000 deaths per year worldwide
- Unfortunately 50% of these are in children under 15yrs
- Mainly Asia and Africa
- Post exposure treatment is available



Methods:

- A reverse transcription loop mediated isothermal amplification (RT-LAMP) assay was designed to detect Classical rabies virus
- The assay is performed at 68°C in a single tube containing both the reverse transcriptase (Thermoscript; Invitrogen) and the polymerase
- The assay was tested using a Genie I machine with enzymology from OptiGene
- Using "tagged" loop primers detection of LAMP products was performed on lateral flow devices
- Samples from Ghana, Morocco, Nigeria, Kenya, Botswana, South Africa, Turkey and Pakistan were tested from various species: human, dog, bat, jackal, mongoose and goat

Results:

- The RT-LAMP was extremely fast using the enzymes from OptiGene with most signals being positive in under 20 minutes
- Using the enzymes from OptiGene produced a faster result than other suppliers assessed
- Temperature annealing analysis post-amplification allows reliable identification of product – confirming a positive result
- Using lateral flow devices to capture tagged LAMP products it was possible to detect positive rabies in dog brain samples from Ghana
- It was possible to detect classical rabies from a wide geographical area covering the two clades of virus (cosmopolitan and Arctic-like)
- RT-LAMP appears to be 1log less sensitive than the RT-PCR¹ currently employed for detection of rabies virus at VLA but takes a fraction of the time

Figure 1: Fluorescence plots of positive and negative classical rabies samples

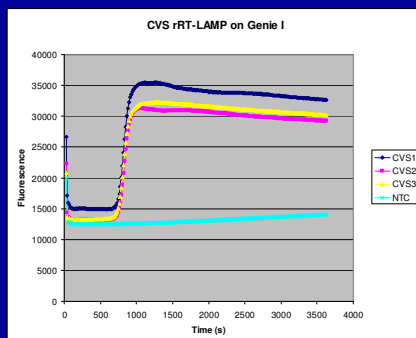
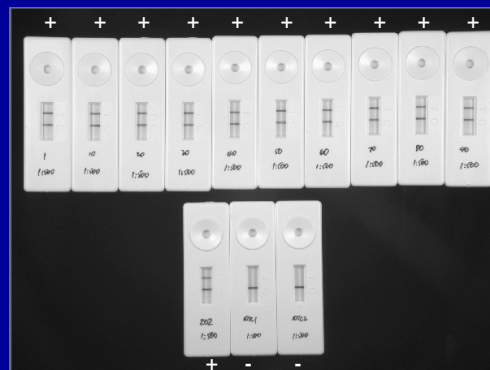


Figure 2: Detection of the LAMP products from 10 Ghanaian positive dog samples using lateral flow devices



Conclusions:

- RT-LAMP produces extremely rapid positive signals using purified rabies RNA from clinical samples and isolated virus
- Using enzymology from OptiGene the rabies assay performs well and a positive signal is produced more rapidly than with other enzymes assessed
- The OptiGene instrument (Genie I) allows for the acquisition of a very large number of data points during the run and annealing analysis is possible confirming the identity of product
- The use of lateral flow devices allows detection of LAMP products in an inexpensive manner

References:

¹ Wakeley, P.R., et al. 2005. Development of a Real-Time, TaqMan Reverse Transcription- PCR Assay for the Detection and Differentiation of Lyssavirus Genotypes 1, 5 and 6. J. Clin. Micro. **43**: 2786-2792.

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