

Development of a LAMP assay to detect the causative agent of strangles



S.E. North, P.R. Wakeley and J. Sawyer

Technology Transfer Unit, Biotechnology Department, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB.
Contact: s.north@vla.defra.gsi.gov.uk

Background:

- Strangles is a highly infectious disease of the upper respiratory tract of horses caused by *Streptococcus equi equi*.
- Infection with *S. equi equi* causes abscesses in the lymph nodes around the throat. The swelling of the lymph nodes may restrict the airways, hence the name strangles. When the abscesses rupture, a highly infective purulent discharge is released.
- Infected horses can be a potential source of infection for up to six weeks after the resolution of clinical signs, others can become a reservoir for infection by becoming periodic asymptomatic shedders.
- The gold standard for testing is microbial culture, but this can be slow and interpretation complicated by the presence of commensal bacteria such as *S. equi zooepidemicus*.

Methods:

- A loop mediated isothermal amplification (LAMP) assay was designed to detect *S. equi equi*.
- The assay was tested using a Genie I machine and enzymology from OptiGene.
- Bacterial DNA was extracted directly from swabs, using a pre-existing extraction method¹
- 92 clinical swabs from horses in the UK with suspected strangles (56 positive and 36 negative (by culture) were tested using the LAMP assay to determine the diagnostic sensitivity of the LAMP assay.
- A confirmatory melt analysis was carried out on the LAMP products. Any differences in the amplified sequence had different melt temperatures.
- The analytical specificity of the LAMP assay was tested using 36 different bacterial species, including equine commensals such as *Rhodococcus equi*, *Oligella urethralis* and *Streptococcus equi zooepidemicus*.
- The analytical sensitivity, inter- and intra-variability of the LAMP assay was determined using target DNA extracted from a positive control strain.

Results:

- A LAMP assay to detect the causative agent of strangles, *S. equi equi*, was successfully designed (Figure 1)
- The LAMP assay had 100% analytical specificity for the target organism and did not cross-react with *S. equi zooepidemicus*
- The positive control could be detected in approximately 12 minutes
- Using culture as the gold standard the diagnostic sensitivities and specificities of the LAMP assay were 88% and 56%, respectively (Table 1)
- The LAMP assay will detect as few as 12 cfu/ul of the target bacteria.
- The inter-assay variation was very low (0.11%)
- The intra-assay variability was also very low (0.14% CV)
- The difference between the melt temperatures of the LAMP products of the positive control (n=16) was 0.5°C (Figure 2)

Figure 1: Fluorescence plots of positive and negative strangles swabs

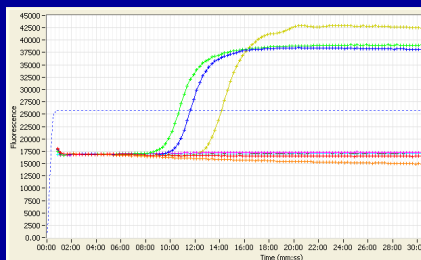


Figure 2: Melt analysis of positive and negative strangles swabs

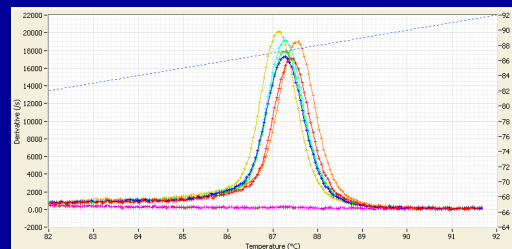


Table 1: Diagnostic sensitivity and specificity

		Culture		
		Pos	Neg	Total
LAMP assay	Pos	49	16	65
	Neg	7	20	27
	Total	56	36	92

Diagnostic Sensitivity = 88%

Diagnostic Specificity = 56%

Conclusions:

- This LAMP assay offers the potential for a quick test for the diagnosis of strangles that is easy to run and interpret.
- A rapid diagnostic assay, such as this, could prove essential in helping control outbreaks and would prove particularly important in the detection and diagnosis of asymptomatic sub-clinical shedders.

References:

¹ Wakeley, P.R., et al. 2006. Development of a real time PCR for the detection of *Taylorella equigenitalis* directly from genital swabs and discrimination from *Taylorella asinigenitalis*. *Vet. Microbiology*. 118, 247-254.

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