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## Assay Performance Characteristics Summary Sheet

**Assay:** Vesicular Stomatitis Virus rRT-PCR for Equine Samples

**Disease:** Vesicular Stomatitis (VS) caused by VSV – New Jersey and Indiana 1 serotypes

**Type of Assay:** rRT-PCR

**Purpose of Assay:**

Detection of VSV in lesion tissues or lesion swabs collected from equine species exhibiting clinical signs compatible with vesicular stomatitis. This assay will only be used in the NAHLN laboratories when the following criteria have been met: 1. Laboratory has been activated during the current VSV outbreak year, 2. Samples are part of a Foreign Animal Disease Investigation, and 3. Samples are from equine species exhibiting signs of vesicular disease from a premises where no other species are showing vesicular signs

Samples from animals of other species with vesicular signs or from premises where mixed species are showing vesicular signs must be sent to FADDL in New York for testing

**Background Information:**

This assay targets the conserved L gene of VSV and is based on Hole et al. 2006 and 2010 with alterations as implemented by FADDL (inclusion of an internal control and change in fluorophore on one of the probes in order to accommodate the internal control) for use in their bovine vesicular testing algorithm. The only change made for this validation was using equine samples.

**Platform(s):** ABI 7500

**Chemistry(ies):** TaqMan® Fast Virus 1-stem Master Mix

**Sample Types:** oral swabs and lesion tissue

**Species:** Equine

**Performance Characteristics:**

**Analytical Sensitivity:**

Using Synthetic Templates		
VSV Serotype	LOD (copies/reaction)	Amplification Efficiency
NJ	1	78-88% <sup>a</sup>
IND-1	13	89-140%

<sup>a</sup>It has been noted that the amplification efficiency estimates for the NJ reaction are low, particularly in the context of the LOD estimate for that same reaction. This lower amplification efficiency estimate could be explained by the fact that there are multiple, energetically favorable secondary structures and self-dimers possible with the NJ primer and probe sequences.

## Performance Characteristics (continued):

### Analytical Specificity:

Analytical specificity was determined by testing for cross-reactivity with other viruses that are genetically similar and those that produce similar clinical disease. All viruses tested were negative for both the NJ and IND-1 serotypes. Viruses included in this analysis: equine herpesviruses 1-5, asinine herpesviruses 1 & 2, equine influenza viruses (Prague/56 & Miami/63), Cache Valley virus, Jamestown Canyon virus, and Rabies virus.

### Repeatability:

Virus	Source of variability	Concentration	Standard Deviation Estimate
NJ	Interassay	---	0.30
		Intrassay	0.05
	Intrassay	High	0.11
		Medium	0.19
IND-1	Interassay	---	0.21
		Intrassay	0.05
	Intrassay	High	0.10
		Medium	0.28

### Diagnostic Performance Characteristics:

Diagnostic specificity and sensitivity were determined by extracting and testing swab effluent and tissue homogenates from the three sample cohorts: Negative cohort, Indiana 1 positive cohort, and New Jersey cohort. For the IND-1 and NJ cohorts, it is important to note that all of the samples tested were part of FAD investigations during VSV outbreaks in the U.S., thus all of the animals were exhibiting clinical signs consistent with vesicular stomatitis at the time of sampling.

		Cohort	Sample Type	Estimate
NJ	Diagnostic Specificity	Negative	Swab	1.00 (95% CI 0.98, 1.00)
		NJ	Swab	0.96 (95% CI 0.93, 0.98)
	Diagnostic Sensitivity	NJ	Tissue	0.94 (95% CI 0.71, 1.00)
			Swab	0.80 (95% CI 0.70, 0.89) <sup>b</sup>
			Tissue	1.00 (95% CI 0.86, 1.00)
IND-1	Diagnostic Specificity	Negative	Swab	1.00 (95% CI 0.98, 1.00)
	Diagnostic Sensitivity	IND-1	Swab	0.93 (95% CI 0.66, 1.00)
			Tissue	0.95 (95% CI 0.82, 0.99)

<sup>b</sup>For the NJ cohort, there were 77 swab samples from disease status positive horses, of which 62 tested positive by the NJ reaction of the VSV PCR. All 15 of the disease status positive, PCR negative samples were CF positive, 14 were cELISA positive, all were VI negative, all were from horses exhibiting clinical signs consistent with VS. These were most likely horses infected with VSV. However, due to the timing, location, or method of swabbing, these horses tested negative on the VSV rRT-PCR. While the negative rRT-PCR results for these samples are reflected in the sensitivity estimate for the NJ reaction of this assay, it is likely that the assay returned negative results because the amount of VSV NJ nucleic acid was below the LOD or there was no VSV NJ nucleic acid present in the samples rather than because it failed to detect nucleic acid that was there.

### References:

1. Hole, et al. Detection and serotype-specific differentiation of vesicular stomatitis virus using a multiplex, real-time, reverse transcription-polymerase chain reaction assay. *J Vet Diagn Invest* (2006) 18:139-146
2. Hole, et al. Improvement and optimization of a multiplex real-time reverse transcription polymerase chain reaction assay for the detection and typing of vesicular stomatitis virus. *J Vet Diagn Invest* (2010) 22: 428-433.