A highly sensitive and specific commercial cELISA for the diagnosis of Foot and Mouth Disease

Foot and Mouth Disease Virus (FMDV) is a single-strand, positive-sense RNA virus in the *Picornaviridae* family. The resulting disease is exceedingly contagious, with high fever and vesicular lesions in cloven hoofed animals including domestic livestock such as cattle, pigs, sheep, and goats. It is of major international concern, with heavy impact on trade economics and extensive regulations governing control. Seven regionally specific, immunologically distinct serotypes have currently been identified. In order to address important challenges in FMD diagnosis, an effective assay must be broadly reactive across viral serotypes and host species, and be able to differentiate between infected and vaccinated animals (DIVA capable).

A commercial competitive ELISA (cELISA) has been developed and validated by VMRD, Inc. in collaboration with the Institute for Infectious Animal Diseases and the Plum Island Animal Disease Center of the US Department of Homeland Security and is in the final stages of USDA licensure. This cELISA detects antibodies against the FMDV 3ABC non-structural protein through inhibition of specific monoclonal antibody binding to a highly immunogenic yet highly conserved epitope within the target protein. This target protein enables the assay to be DIVA capable, as it is only present in replicating virus and not in purified vaccines. The cELISA format enhances specificity while offering the flexibility of multispecies use. Thus far, this assay has been validated for cattle, pigs, and sheep.

An optimal cutoff of 40% inhibition was determined. Serum samples of known infection status were evaluated from 503 FMDV negative cattle of US origin (FMDV-free), 121 cattle experimentally infected with various FMDV isolates representing all 7 serotypes, 117 naturally infected cattle from Cameroon and South Africa, and 52 vaccinated cattle later challenged with live FMDV.

		Known Infection Status		
		+	-	Sum
VMRD cELISA	+	239	6	245
	-	1	643	644
	Sum	240	649	889
Sensitivity: 99.6% Specificity: 99.1%				

	VMRD cELISA	Current kit used by USDA
Sensitivity	**99.6%**	96.7%
Specificity	**99.3%**	97.9%

A subset of these samples (n=386) was also run for comparison on another commercial non-structural protein ELISA that is currently utilized by the USDA and considered to be the most specific assay on the market at this time.

Results demonstrate the **broad serotype reactivity** of the VMRD cELISA, with identification of antibody to all seven serotypes represented in experimental infections and five serotypes in naturally infected cattle.

DIVA capability was confirmed by negative test results in vaccinated, unchallenged cattle that then became positive after challenge, with the VMRD cELISA detecting more positives than the other commercial assay in this challenge group.

In unvaccinated, experimentally infected animals, the VMRD assay identified **seroconversion in all by 15 days post-infection**, with comparatively delayed detection of seroconversion by the other commercial assay.

# (+) on test/ # true positive	Post-infection	
	13-15 days	21-29 days
VMRD cELISA	**89/89**	**28/28**
Current kit used by USDA	81/89	28/28

The broad reactivity, DIVA capability, and high sensitivity and specificity shown in this study demonstrate the ideal nature of this new assay for use in both US and international FMDV control programs.

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