



United States  
Department of  
Agriculture

Marketing and  
Regulatory  
Programs

Animal and Plant  
Health Inspection  
Service

Veterinary Services

National Veterinary  
Services Laboratories

National Animal Health  
Laboratory Network

P.O. Box 844  
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Ames, IA 50010  
(515)663-7731  
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## Assay Performance Characteristics Summary Sheet

**Disease:** Foot and Mouth Disease Virus

**Assay:** Antibody Test Kit

**Type of Assay:** cELISA

**Purpose of Assay:** This assay is intended to provide results which will give guidance about the presence of FMDV infection.

**Background Information:** Sample serum antibodies to FMDV inhibit the binding of a horseradish peroxidase (HRP)-labeled monoclonal antibody to the FMDV antigen coated on the plastic wells of the assay plate. Binding, or lack of binding, of the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent color development. Strong color development indicates little or no blockage of HRP-labeled monoclonal antibody binding and therefore the absence of antibodies to FMDV in the sample serum. Weak or no color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of FMDV antibodies in the sample serum.

**Platform(s)/Equipment:** Materials required but not included in the kit include: Single and multichannel adjustable-volume pipettors and disposable plastic tips, non-antigen-coated transfer plates, ELISA microplate absorbance spectrophotometer with 450 nm filter, timer, multichannel pipettor reservoirs, wash bottle, manual multichannel washing device or automatic plate washer.

**Chemistry(ies)/Reagents:** Antigen coated plates, Positive Control, Negative Control, 100X Antibody-Peroxidase Conjugate, Conjugate Diluting Buffer, Serum Diluting Buffer, 10X Wash Solution Concentrate, Substrate Solution, Stop Solution

**Sample Type(s):** serum

**Species:** bovine, swine and sheep

**Performance Characteristics**

**Analytical Sensitivity:** Not Available

**Analytical Specificity:** Not Available

**Analytical Repeatability:**

The negative control was run in 400 wells from 5 plates.

**Intra-assay:**

<b>Plate 1: Mean OD: 1.056</b>	<b>Standard Deviation: 0.032</b>	<b>CV: 3.1%</b>
<b>Plate 2: Mean OD: 0.977</b>	<b>Standard Deviation: 0.041</b>	<b>CV: 4.2%</b>
<b>Plate 3: Mean OD: 1.007</b>	<b>Standard Deviation: 0.030</b>	<b>CV: 3.0%</b>
<b>Plate 4: Mean OD: 1.064</b>	<b>Standard Deviation: 0.036</b>	<b>CV: 3.4%</b>
<b>Plate 5: Mean OD: 1.149</b>	<b>Standard Deviation: 0.039</b>	<b>CV: 3.4%</b>

**Inter-assay:**

<b>Mean Negative Control OD 5 plates: 1.051</b>	<b>Standard Deviation: 0.066</b>	<b>CV: 6.2%</b>
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**Multi Lab:** A serum panel, consisting of 16 samples and the kit positive and negative controls, was run in replicate by two external laboratories (three independent runs per laboratory). The average percent CV of the replicate samples was 3.1%.

**Diagnostic Sensitivity: 99.6% (bovine)**

**Diagnostic Specificity: 99.1% (bovine)**

### Assay performance in various samples

#### Bovine samples

- 240 positive samples tested
  - VMRD identified 239 as positive (99.6% sensitivity)
  - Priocheck identified 232 as positive (96.7% sensitivity)
- 146 negative samples tested
  - VMRD identified 145 as negative (99.3% specificity)
  - Priocheck identified 143 as negative (97.9% specificity)

VMRD additionally tested a total of 649 negative samples and identified 643 as negative (99.1% specificity). Tested 278 field samples from Mongolia (O type) Species included were Bovine (15 Ovine (84 samples), Caprine (28 samples) and Wild Gazelle (10 samples). 100% concordance of results with Priocheck reference assay on 167 positives and 111 negative samples

#### Caprine samples

- 5 positive samples tested and all were correctly identified (100% sensitivity)
- 53 negative samples tested and all were correctly identified (100% specificity)

#### Porcine samples

- 207 negative samples tested
  - VMRD identified 207 as negative (100% specificity)
  - Priocheck identified 207 as negative (100% specificity)
- 65 positive animals tested
  - VMRD identified 63 as positive (96.9% sensitivity)
  - Priocheck identified 50 as positive (76.9% sensitivity)

We excluded 35 field samples from Africa from this study as we did not know the infection status animals and both the tests called them negative

#### Ovine samples

- 151 negative samples tested
  - VMRD identified 149 as negative (98.6% specificity)
  - Priocheck identified 151 as negative (100% specificity)
- 63 positive samples tested
  - VMRD identified 48 as positive (76.1% sensitivity)
  - Priocheck identified 43 as positive (68.2% sensitivity)