



SpinCon® Based Air Sampler  
Foot and Mouth Disease Virus (FMDV)  
Test Report

Conducted at the  
Agricultural Research Service  
Plum Island Animal Disease Center Facility  
Plum Island, NY  
9-11 July 2001

By: Steven P. Medaglia  
Chief Operating Officer  
Lares Corporation

8301 State Line Road, Kansas City, MO 64114  
Main: 816-360-3895 or 888-450-4321, Fax: 816-931-2451  
website: [www.sceptorindustries.com](http://www.sceptorindustries.com)

## **Abstract**

Employment of a high volume air sampling technology in combination with Polymerase Chain Reaction analysis could shorten the time necessary to identify the presence of aerosolized Foot and Mouth Disease (FMDV). This concept was tested under sponsorship of the Agricultural Research Service at the Plum Island Animal Disease Center.

A SpinCon® based air sampler was used to collect FMDV virus particulates respirated by two infected pigs over a three-day period. Sample collection times of 5, 10, 30-minutes, 2-hours, and 4-hours were run following the establishment of both negative and positive controls for the experiment. In addition, replicated field decontamination procedures were successfully tested to reduce the probability that a sample set was contaminated from residual FMDV particulates from the previous collection cycle. The collected samples were analyzed using a RAPID PCR device. This combination of technologies successfully collected and identified the FMDV at very low aerosolized concentration levels.

## **Introduction**

Foot and Mouth Disease presents a significant threat to the livestock industry of the United States. Current identification procedures involve the collection and analysis of sera from suspect animals. Research personnel at the Agricultural Research Service at the Plum Island Animal Disease Center expressed interest in a method proposed by Lares Corporation in May 2001 for collecting aerosolized viral particulates. Employment of this technology could shorten the time to identify the presence of the disease. The objective of this proposal was to use a high volume, SpinCon® based air sampler<sup>1</sup> in conjunction with a TaqMan based, real-time Polymerase Chain Reaction (PCR) assay (run in a RAPID<sup>2</sup> thermocycler), to collect and identify the FMDV. This proposal was predicated on the belief that FMDV was respirated by infected animals and could travel in an aerosolized state to other locations. Confirmation of this collection/identification capability would first be proved in a laboratory environment at Plum Island, and with proven success, be continued in a field environmental test. Lares was invited to return to Plum Island to conduct the laboratory testing during the week of 9-11 July 2001.

<sup>1</sup>The SpinCon® technology was developed and patented by Midwest Research Institute. Lares/Camber holds an exclusive patent license for the sale of the SpinCon® for use in production systems.

<sup>2</sup>The RAPID is produced by Idaho Technology

## Experimentation

### *Equipment*



Figure 1 – PAS 450/10

Figure 1 shows the Portable Air Sampler (PAS) 450/10 SpinCon® based air sampler. This version operates at a flow rate of 450 liters per minute and deposits a 10ml liquid sample size into a collection bottle at the end of the run cycle. The system operates on 120 volts AC or 12/24 volts DC. It weighs approximately 46 pounds and is fully portable. The dimensions of the unit are : 18” high x 15” wide x 8” deep.



Figure 2 – RAPID

Figure 2 shows the RAPID Polymerase Chain Reaction analytical device. It is a field-hardened rapid thermocycler with concurrent fluorescence monitoring. It is capable of automatically analyzing samples for the presence of specific nucleotide sequences. The RAPID weighs approximately 50 pounds and can fit into a backpack for transport under a variety of environmental conditions. The system can process up to 32 samples using freeze dried reagents, with analysis completed in less than 30 minutes. Up to 8 pathogens in 20 micro-liter sample size can be identified simultaneously. The RAPID features a remote reach-back capability that allows real-time monitoring of a reaction from a remote location via a standard web browser. System dimensions are 19.4” long x 14.3” wide x 10.5” deep.

## ***Methodology***

The testing team consisted of Steve Medaglia, Lares Corporation; Darrel Sprague, Midwest Research Institute; Frank Roberts, CRE, Inc.; Dr. Dan Rock, Dr. Manuel Borca, Dr. Tony Ramirez, Mr. Bill Lang, and Mr. Edan Tulman, Agricultural Research Service, Plum Island. The aerosol collecting was done in animal pen #1136, with the PCR analyses of the collected samples completed in laboratory Mod 1.

Steve Medaglia, Darrel Sprague, and Frank Roberts operated the air sampler, collected aerosol samples, and recorded raw data over the three-day period. Dr. Borca and Dr. Ramirez injected the two pigs with FMDV, monitored their state of health and took nasal swabs for analysis. Mr. Tulman processed the collected samples and ran the PCR analysis. Mr. Lang assisted in the PCR analysis while Dr. Rock provided overall supervisory guidance to the group.

Work began at 1022 hours, 9 July 2001, and concluded at approximately 1600 hours, 11 July 2001. Personnel would work in the laboratory from approximately 0900 hours to 1600 hours. These times were driven by the ferryboat schedule to and from Plum Island. No personnel remained overnight to conduct continuous sampling and monitoring.

The sample collection media was a Phosphate Buffer Saline (PBS) solution consisting of PBS, 0.1% Triton x-100, 0.04% Kathon, 0.22um sterile filtered at a pH in the range 8.0 to 8.3. In order to counter the effects of evaporation and maintain a constant 10ml fluid level, sterile water was periodically injected into the air sampler collection chamber.

Prior to setting up the air sampler in animal pen #1136, 10ml each of PBS and sterile water were tested for the presence of FMDV. Both controls indicated negative. Another 10ml sample of PBS was provided and spiked with approximately  $10^6$  particles of FMDV. This sample was analyzed, indicated positive, and provided the positive control as a reference for later PCR testing.

The air sampler was set up in pen #1136, approximately 40.5" above the floor and behind a grating to prevent interference by the animals (Figure 3). The room measured approximately 10' wide x 8' long x 15' high. A 110v outlet located in the pen provided power to the sampler. A digital thermometer/relative humidity indicator was set up near the viewing window to provide Temp/RH data at the time of sample collection.

Two piglets were provided as test subjects. Prior to inoculation with the FMDV, a 30-minute air sample was



Figure 3 - Air Sampler in Pen #1136

taken to provide another negative control. This sample was analyzed and provided a negative indication. The pigs were inoculated at approximately 1200 hours, 9 July 2001. One was injected under the tongue (pig #46), while the other (pig #44) was injected between the toes. Immediately following inoculation, a 30-minute sample was taken. This sample indicated a positive for FMDV.

Following this initial post-inoculation collection, the following methodology was employed: 9 July (PM) – 30-minute sample; 10 July (AM) – decontamination of the sampler collection path, 30-minute sample; 10 July (PM)/11 July (AM) - decontamination of the sampler collection path, 5-minute sample, 10-minute sample and 30-minute sample. One 4-hour sample was taken during the overnight period on 9 July, and another 2-hour sample was taken at the end of the day on 11 July. Following each collection cycle, the samples were placed in a stainless steel container that was externally decontaminated prior to removal to lab Mod 1. On 9 July, for samples #5-10, no ice was used in these transport containers. Due to the relatively fragile nature of the FMDV, it was decided to use ice in the containers for all subsequent samples. Within lab Mod 1, the sample bottles were removed from the containers and placed on ice prior to processing and analysis. PCR assays performed in the RAPID used approximately 3.5 microliters of unextracted, raw liquid directly from the sample bottle or 3.5 microliters of extracted liquid (10 microliter equivalent of unextracted liquid) that was processed using a Qiagen RNA prep kit. In all



Figure 4 – RAPID PCR Analysis in Lab Mod 1<sup>3</sup>

but one sample case, the PCR analysis was done within 30-60 minutes of the samples being brought back to lab Mod 1 (Figure 4). The exception was the 4-hour sample that sat in the collection bottle, during the overnight period, for approximately 13.5 hours before it was processed. Additional confirmatory assays were run on the stored samples on both 12 and 13 July (24-48 hours following the last collection).

During the three-day test period, the pigs were sampled twice daily with nasal swabs for virus isolation. The temperature of the pigs and the presence of lesions were recorded daily. A fever was considered any temperature above 104°F. Lesions were the presence of vesicles of any size.

Decontamination was conducted using the following procedure:

- Wipe exterior of case, air inlets, handles, water/PBS bags, external hoses and platform around sampler with 10% bleach solution
- Run 10% bleach solution through two rinse cycles. Cover intake port with bleach soaked wipe. Allow to stand in system for 10 minutes
- Conduct two rinse cycles with sterile water following 10 minute wait time

<sup>3</sup>From left to right: Edan Tulman (ARS), Manuel Borca (ARS), Steve Medaglia (Lares), and Darrel Sprague (MRI)

- Conduct third rinse cycle with sterile water and collect sample for testing
- Run PCR analysis on rinse water sample to determine status of decon

In all four decontamination cycles, the collected rinse indicated negative for FMDV.

## Results

The following tables synopsize the data collected and analyzed during the test

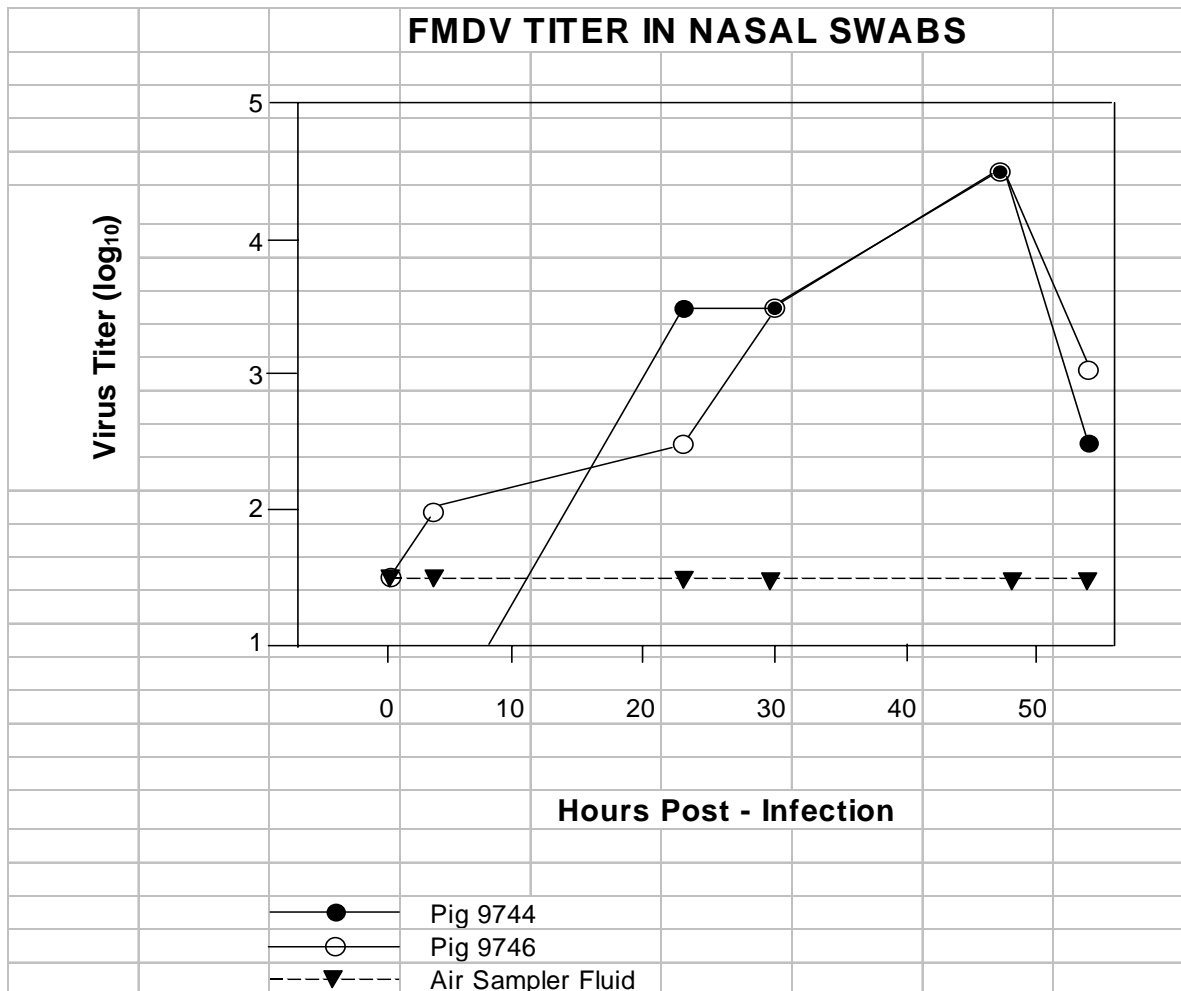


Table 1  
Approximate Titer Concentration

**Table 2. Air Sampler FMDV Experiment Summary**

	Extracted Samples (Qiagen RNA Prep Kit)			Unextracted Samples	
	<u># samplings</u>	<u>positive assays/total<sup>4</sup></u>	<u>estimated number of molecules<sup>1</sup></u>	<u>positive assays/total</u>	<u>estimated number of molecules*</u>
<b>Negative solution controls</b>	2	0/11 (0%)			
<b>Pre-exposure air sample</b>	1	2/15 (13%) <sup>2</sup>	(1)		
<b>Post-decontamination rinses</b>	4	0/10 (0%)		0/6 (0%)	
<b>Total Positive</b>		2/36 (6%)			
<b>FMDV positive control (10e6 particles)</b>	1	12/12 (100%)	777000-1097000		
<b>Post-exposure air samples</b>					
<b>5 min air sample</b>	2	5/5 (100%)	380-2789	3/3 (100%)	94-205
<b>10 min air sample</b>	3	7/7 (100%)	196-13290	3/4 (75%)	0.4
<b>30 min air sample</b>	5	21/21 (100%)	3-8573	4/4 (100%)	46-51
<b>2 hr air sample</b>	1	3/3 (100%)	2160-3514	2/2 (100%)	1585-1636
<b>4 hr/overnight air sample</b>	1	0/4 (0%) <sup>3</sup>		1/2 (50%)	
<b>Total Positive</b>		36/40 (90%)		13/15 (87%)	

<sup>1</sup> Estimated number of template molecules only for samples run with standard curve.  
genomes = #molecules  
infectious unit = 1000 molecules

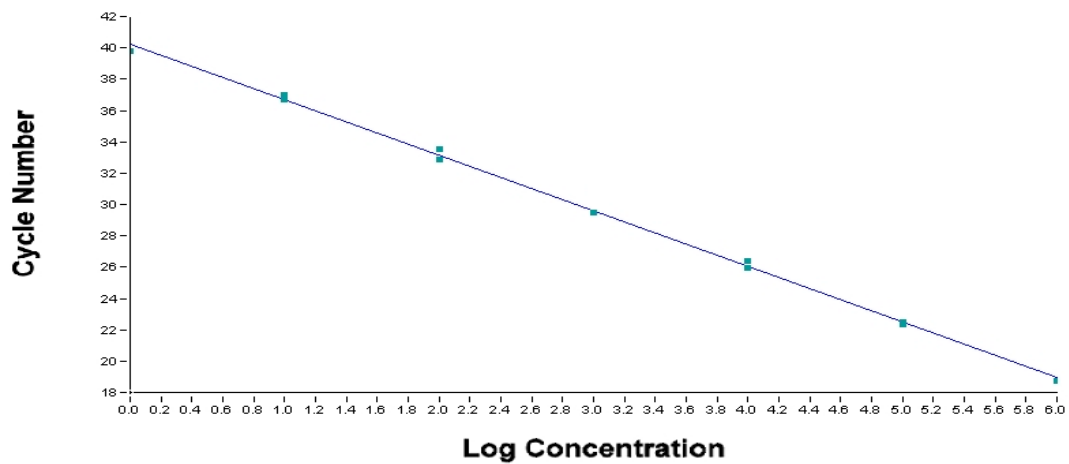
The molecular count was estimated using the following methodology: the number of template genomes was derived from a standard curve generated with dilutions of experimental sample #4 (RNA extracted from collection buffer spiked with FMDV-infected cell culture supernatant). The titer accounts for an approximate sup titer of 10e8 infectious units/ml, and assumes a total viral particle titer of approximately 1000x sup titer (10e11 particles/ml).

<sup>2</sup> The positive indications (2/15) for the pre-exposure air sampling may have been due to contamination of the sample during handling in the laboratory. During PCR analysis on 9 July when the sample was first taken, and during subsequent assays run on 10 and 11 July, the sample was negative for FMDV. Two assays run on 12 July showed one negative, then one positive indication. Four more assays were run on 13 July, with one positive and three negative indications. Mr. Tulman stated that the positive indications were very weak on the PCR display and he felt they were due to trace contamination introduced during handling.

<sup>3</sup> The 4-hour sample, when analyzed as extracted RNA, was negative. One of the raw sample assays indicated positive, but subsequent assays (extracted, unextracted and re-extracted) failed to give a positive signal. The positive indication may have been due to trace contamination during handling. Although we expected positive assays following the overnight collection, we speculated that the negative results were due to viral instability at room temperature in the collection fluid during the 13.5 hour delay in processing.

<sup>4</sup> Assay=individual PCR reaction

Crossing Points ■ ■ ■ ■ ■  
 Linear Regression —



Baseline Adjustment: Arithmetic	Noise Band Cursor: 8.4112	Analysis Method: Fit Points	Number of Fit Points: 2
Crossing Line: 8.4112	Slope: -3.554	Intercept: 40.24	Error: 0.688
Color Compensation: Off	r: -1.00		

Table 3  
 Standard Curve Used in Concentration Analysis

## LightCycler Quantification Report

LC Run Version: RAPID Run 1.2.8

LCDA Version: 3.1.106

This experiment was run on July 13, 2001 by All Users.

### Sample Information

Rotor Position	Sample Name	Rep Of...	Sample Type*	Known Cone	Calculated Cone. (Units)	Crossing Points	Sample Comments
1	1		U				FMDV ☐☐
2	2		U				FMDV ☐☐
3	4		S	1,000,000	1,108,000	18.76	FMDV ☐☐
4	4a		U		15,230	25.38	FMDV ☐☐
5	4-1		S	100,000	106,800	22.37	FMDV ☐☐
6	4-1		S	100,000	100,700	22.46	FMDV ☐☐
7	4-2		S	10,000	7,964	26.38	FMDV ☐☐
8	4-2		S	10,000	10,360	25.97	FMDV ☐☐
9	4-3		S	1,000	1,087	29.45	FMDV ☐☐
10	4-4		S	100.0	116.9	32.89	FMDV ☐☐
11	4-4		S	100.0	75.63	33.57	FMDV ☐☐
12	4-5		S	10.00	8.200	36.99	FMDV ☐☐
13	4-5		S	10.00	9.869	36.71	FMDV ☐☐
14	4-6		S	1.000	1.308	39.83	FMDV ☐☐
15	5		U		54.24	34.08	FMDV ☐☐
16	5.1		U		2.675	38.72	FMDV ☐☐
17	6		U		18.93	35.70	FMDV ☐☐
18	7		U		0.838	40.52	FMDV ☐☐
19	7.1		U				FMDV ☐☐
20	8		U				FMDV ☐☐
21	9		U		202.4	32.05	FMDV ☐☐
22	10		U		224.4	31.89	FMDV ☐☐
23	12		U				FMDV ☐☐
24	13		U		2,707	28.04	FMDV ☐☐
25	14		U		5,738	26.89	FMDV ☐☐
26	15		U		8,612	26.26	FMDV ☐☐
27	16		U				FMDV ☐☐
28	17		U		374.5	31.10	FMDV ☐☐
29	18		U		833.3	29.86	FMDV ☐☐
30	19		U		354.4	31.18	FMDV ☐☐
31	20		U				FMDV ☐☐
32	21		U		3,676	27.57	FMDV ☐☐

\*P=Positive, U=Unknown, N= Negative, S=Standard, < > = De-Selected

Table 4  
Quantification Report – Calculated Concentrations Based on Standard Curve

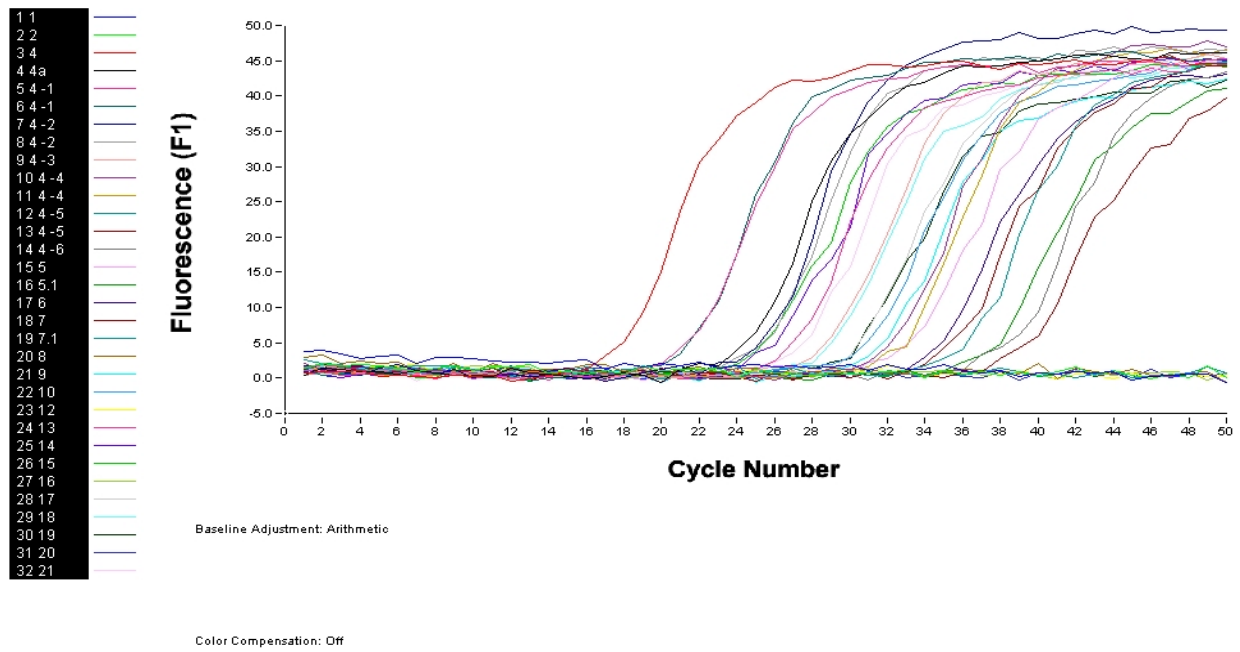


Table 5  
 Graphic Display of PCR Analysis

Temperature (°F)	Day 1	Day 2	Day 3
Pig #44	101.8	105.2	104.4
Pig #46	102.6	104.4	103.3

Table 6  
 Recorded Temperature of Pigs

<b>Lesions</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>
Pig #44	None	1 - Hind leg	1 - Hind leg 2 - Forelegs 2 - Nose
Pig #46	None	1 - Hind leg 2 - Forelegs	2 - Hind legs 2 - Forelegs 2 - Tongue

Table 7  
Lesions



Figure 5  
Day 3 –Infected Pigs in Pen #1136

## Discussion

During preliminary discussions prior to testing, it was not known how soon after initial infection one could expect to detect the presence of the virus in aerosolized form. You would not normally expect to collect and detect the virus in the first 30-minute sample. One possible explanation for the positive indication is that there may have been fluid leakage around the injection site of pig #46, which was under the tongue, and the FMDV may have then been aerosolized when the pig was snorting.

FMDV is one of the smallest viral particulates known (~ 20 nanometers), and is extremely fragile. Additionally, Table 2 illustrates the relative titer concentration levels in the sera of the infected pigs versus the low level, estimated concentrations in the collected sample (near or below threshold of an infectious dose). While the results of this test were conducted under laboratory conditions, they are a testimony to the capability of the SpinCon® technology to collect very low level concentrations of an extremely small, aerosolized pathogen and preserve it long enough for analysis and identification.

The time line to identify the virus using the combination of SpinCon and RAPID reduced the visual symptom identification time by at least 24-36 hours. Potential implications of the success of the test include:

- The ability to determine the actual presence of the virus in order to make decisions for controlling its spread (vaccination vs. slaughter and assist in determining where decontamination and access control procedures may be necessary).
- Real time mapping of viral spread. Potentially reduce the economic impact of unnecessary animal slaughter
- Confirmation that the virus is not present on goods being transported from an infected area.

The next step would be to conduct actual environmental field tests in infected areas to verify these implications.

## **Conclusions**

Lares' main objective in conducting this test was to demonstrate that the FMDV could be collected in aerosolized form by the SpinCon® based air sampler and identified using the RAPID PCR analyzer. This objective was clearly achieved and at very low concentration levels. In addition, it was also demonstrated that a field decontamination of the fluid and air path could be conducted to reduce the chances for contamination in subsequent sample collection.



The ability to collect an aerosolized form of the FMDV that was respired from an infected animal and positively identified at least 24 to 36 hours prior to the onset of visible symptoms (lesions) provides a valuable tool to agricultural agencies in their fight against this highly infectious, endemic disease.

## Appendix A

### **Points of Contact:**

#### *Agricultural Research Service*

Dr. Daniel L. Rock  
USDA ARS Plum Island Animal Disease Center  
P.O. Box 848  
Greenport, NY 11944  
(631) 323-3330

#### *Sceptor Industries, Inc*

8301 State Line Road  
Kansas City, MO 64114  
Phone: 816-360-3895 or 888-450-4321  
Fax: 816-931-2451

See Sceptor's website for additional company information, products, pricing, and a list of authorized independent manufacturer's representatives: [www.sceptorindustries.com](http://www.sceptorindustries.com)

#### *Midwest Research Institute*

Mr. Darrel G. Sprague  
Midwest Research Institute  
425 Volker Blvd  
Kansas City, MO 64110  
(816) 753-7600

#### *Critical Response Engineering, Inc.*

Mr. Frank Roberts/Mr. Pat Minix  
CRE, Inc.  
1600 Prince Street  
Suite 613  
Alexandria, VA 22314  
(703) 299-0252