



# Development of Rapid and Inexpensive Diagnostic Kits for Foot-and-Mouth Disease and Rift Valley Fever

Francisco Monge-Navarro<sup>1</sup>, James F. Papin<sup>1</sup>, Paulo H. Verardi<sup>1</sup>, Leslie A. Jones<sup>1</sup>, and Tilahun D. Yilma<sup>1,2</sup>

<sup>1</sup>International Laboratory of Molecular Biology for Tropical Disease Agents, School of Veterinary Medicine,

<sup>2</sup>Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, California 95616



## Development of a Rapid and Inexpensive Diagnostic Kit for Foot-and-Mouth Disease

### Background

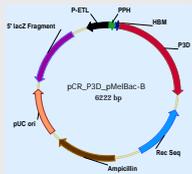
Foot-and-mouth disease (FMD) is one of the most economically devastating vesicular diseases of livestock. Rapid detection of FMD virus (FMDV) infection is essential for prompt identification and prevention of the spread of an outbreak. We are currently developing a rapid diagnostic kit for detection of FMDV. This kit is based on a chromatographic strip test and can be used in the field by technicians. The FMDV antigen can be detected from a drop of blood or serum sample from suspected animals in just a few minutes. For the detection of the viral antigen, specific monoclonal antibodies to the viral RNA polymerase (P3D protein) will be used. Since this protein is highly conserved among all seven types of FMDV, a single kit will be able to detect all FMDV serotypes.

**Objective:** Provide a pen-side test kit for rapid diagnosis of FMD

### Development of the rapid test kit

#### Recombinant FMDV-3D protein

Recombinant FMDV-3D protein was developed to detect anti P3D monoclonal antibodies. The gene for the FMDV-3D protein was subcloned into the baculovirus transfer vector pMelBac. The resulting pMelbacFMDV3D was co-transfected with Bac-N-Blue baculovirus expression system.



Recombinant candidates were confirmed by PCR analysis. Virus was then amplified and High Five insect cell cultures infected to produce baculovirus-expressed FMDV-3D recombinant protein.

Guinea pig anti-FMDV sera was used to confirm antibody reactivity to the recombinant FMDV-3D protein by western blot analysis.

#### bFMDV-P3D Western Blot



bFMDV-3D protein is ~52 KDa

1<sup>st</sup> AB: Guinea pig  $\alpha$ -FMDV 1:1000 2<sup>nd</sup> AB: Goat  $\alpha$ -Guinea pig/AP 1:5000

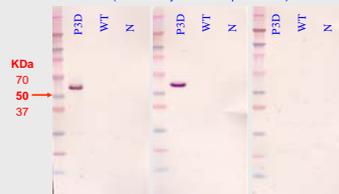
#### Monoclonal antibodies to FMDV-3D protein

The P3D gene of FMDV was subcloned into the vector pVAX1. The resulting plasmid pVAX1P3D was amplified and used as a DNA vaccine to immunize (prime) groups of BALB/c and C57BL/6 mice.

Ni-NTA purified 3D protein was used to immunize mice, before collection of splenocytes to generate hybridomas. Monoclonal antibodies were detected using baculovirus-expressed recombinant 3D protein in ELISAs and western blots.

#### Western Blot of anti-P3D Mab's

(Selected hybridoma supernatants)



bFMDV-3D protein is ~52 KDa

1<sup>st</sup> AB: Hybridoma supernatant 2<sup>nd</sup> AB: Rabbit  $\alpha$ -Mouse/AP 1:5000

The monoclonal antibodies will be tested to confirm that they recognize all 7 types of FMD before being used as reagents to develop the FMDV antigen capture chromatographic strip test.

Validation of the FMDV-3D antigen detection strip test with samples from cattle, sheep, goats and pigs will be conducted at the PIADC.

## Development of a Rapid and Inexpensive Diagnostic Kit for Rift Valley Fever

### Background

Rift Valley fever virus (RVFV) infects most mammalian species including humans, causing high morbidity and mortality. The virus is transmitted and maintained by numerous mosquito genera including several native to North America (Aedes, Culex, and Anopheles). If RVFV is accidentally or deliberately introduced into this country, the disease is very likely to become endemic in North America. In addition, this virus has enormous potential to be used as a bioterrorist agent. In the event of an outbreak of Rift Valley fever in the US, the availability of a rapid diagnostic kit will facilitate the control of the disease. We have expressed the N protein of RVFV in a baculovirus expression system and are using this reagent to develop an indirect ELISA for the detection of RVFV antibodies. The recombinant RVFV N protein has been produced in large quantities and optimized procedures for purification of the protein worked out. The protein is bound specifically by antibody to RVFV in Western blots.

**Objective:** Provide an ELISA kit for rapid diagnosis of Rift Valley fever

### Development of the ELISA test kit

#### Recombinant RVFV N protein

The gene for the RVFV-N protein was subcloned into the baculovirus transfer vector pVL1393. The resulting pVL1393RVFVN was co-transfected with Bac-N-Blue baculovirus expression system.



Recombinant candidates were confirmed by PCR analysis. Virus was then amplified and Sf9 cell cultures infected to obtain baculovirus-expressed RVFV-N recombinant protein.

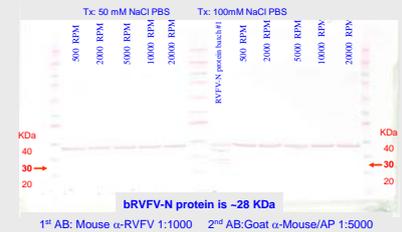
#### Antibodies to RVFV

Groups of mice were vaccinated IP twice with 10<sup>6</sup> PFU of the RVFV MP-12 vaccine strain. Blood was collected at day 45 and serum obtained and pooled. These sera were used to confirm specific antibody binding to the recombinant RVFV-N protein using western blot analysis.

#### Standardization of RVFV-N ELISA

To optimize reagent concentrations, a checkerboard titration was performed using the recombinant RVFV-N protein as solid phase antigen.

#### bRVFV-N Western Blot



#### ELISA Pos/Neg ratio

Sera	RVFV-N antigen per well				
	200 ng	100 ng	50 ng	25 ng	12.5 ng
1:50	7.3	6.8	5.6	3.5	2.9
1:100	6.2	4.5	3.3	2.2	1.8
1:200	3.5	2.8	1.6	1.5	1.4
1:400	1.4	2.3	1.0	1.4	1.2

The ratio of the absorbance from positive sera to negative sera was used to determine optimal antigen concentration for the ELISA.

Validation of the RVFV N ELISA antibody detection test kit using serum samples from cattle, sheep and goats will be conducted in collaboration with UTMB.

#### Acknowledgements

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