RESEARCH REPORT
SWINE HEALTH

Title: A Field-Deployable Fluorescence-Based Sensor Excited by an Organic Light Emitting Device for PRRSV Detection **– NPB #04-185**

Investigator : Louisa B. Tabatabai

Institution: National Animal Disease Center, Ames, Iowa

Date Received: July 7, 2006

Abstract:
The final report describes the research results for the “proof of concept” approach to the development of a sensitive and specific diagnostic test for detecting PRRSV antigen using a novel technology based on a photoluminescence-based sensor as proposed in the original proposal. Because significant problems developed using a new laser dye with the sensor, only preliminary application data are described for the sensor. We used fluorescence-based and colorimetric-based enzyme-linked immunosorbent assays (ELISAs) to detect PRRSV antibodies in sera, PRRSV antigens in tissues. The PRRSV strain used in this study was the NADC-8 strain cultured in Marc-145 monkey kidney cells and purified by precipitation and ultracentrifugation. PRRSV antigen was characterized by gel electrophoresis and Western blotting using monoclonal antibody 15E (K. Platt). Monoclonal antibody SDOW-17 gave different results depending on the SDS-PAGE buffer system used for Western blotting. Additionally we characterized the PRRSV antigen also by enzyme-linked immunosorbent assay (ELISA) using a fluorescein labeled anti mouse IgG conjugate, by western blotting using a horseradish peroxidase-labeled anti-mouse IgG conjugate and by enzyme immunoassay using a ruthenium-anti mouse IgG conjugate. A ruthenium anti-pig IgG was prepared for detection of PRRSV antibody in pig sera and in matching tonsil tissues. Ru-IgG detection using the OLED device produced significant relative photoluminescence signals using the back-detection mode. In addition to the photoluminescence signal, a strong absorption band at 480 nm was also detected. We will continue to optimize detection of PRRSV protein antigen in serum and tissues spiked with PRRSV protein antigen, and we will examine additional sera and tissues from experimentally infected pigs and samples from field cases using both the OLED and fluorometer detection systems.

Introduction

Detection of swine pathogens and specific antibodies is expensive, laborious, requires sophisticated laboratory equipment and considerable technical skill. The goal of this research project is to provide a "proof of concept" for the application of a novel technology to swine diagnostics. The novel technology is a sensor that has potential to be compact, field-deployable, user friendly, sensitive, and inexpensive. We have proposed to use PRRSV as the test pathogen to demonstrate the potential of this new sensing technology.

Objectives

The objectives of the proposed research were:

(1) Design and construct the photoluminescence-based sensor for detection of PRRSV antigen, nucleic acid, and antibody
(2) Evaluate sensitivity and specificity of the sensor utilizing PRRSV-spiked samples
(3) Evaluate the sensitivity and specificity of the sensor on tissue samples collected from pigs experimentally infected with PRRSV.

In order to optimize the sensor-based assays, data obtained from optimized fluorometry-based assays are used and adapted. Because unanticipated problems were encountered with the new laser dye, ruthenium bipyridyl, we decided to continue to use the fluoremetry-based assays for PRRSV detection, while resolving the sensor-based problems. Preliminary data are shown with the sensor, and we will continue to develop the sensor-based assays for PRRSV detection.

Material and methods

PRRSV strain, culture, and isolation. PRRS virus strain NADC-8 was cultured in monkey kidney cells (MARC-145) (1). PRRSV was isolated from 360 mL volume of culture (2) as follows. The procedure involved precipitation of kidney cells in the presence of 1M Zn acetate; the pH was adjusted to 7.2 with 1 M NaOH, and the suspension was stirred at 5°C for 1 hr. The suspension was centrifuged for 30 min at 10,000 x *g* at 5°C and the pellet collected.

The pellet was suspended in 1/50 of original culture volume with saturated EDTA, pH 7.2 and centrifuged at 10,000 x *g* for 15 min. The supernatant was layered onto a 20% w/v cushion of sucrose and then centrifuged at 100,000 x g for 3 h at 5°C in a SW32 Ti rotor. The supernatant was decanted and the pellet containing virus was resuspended in 0.5 mL 10 mM phosphate buffered saline, pH 7.2.

The virus was aliquoted into 100 µL quantities and frozen at -80°C. Total protein was assayed using the Folin phenol reagent (3).

Electrophoresis and Western blotting: SDS-PAGE was done in a 4-12% gel, or in a 16% homogeneous gel. PRRSV was denatured in sample buffer under reducing conditions and 25 µL containing10 µg protein was loaded in each well. Molecular weight standards were from Invitrogen[1]. Gels were stained with Coomassie Brilliant blue R-250 and destained in methanol acetic acid (4). Western blots (5) were blocked with 5% milk in 0.1 M phosphate-buffered saline, pH 7.2-0.05% Tween 20[2] (PBS-Tween) for 15 min and incubated overnight on a rocker at 5°C with a 1:50 dilution of monoclonal antibodies to nucleocapsid protein[3] (15A, 15B, 15C, 15D, 15E, 15E-serum free) in PBS-

[1] Invitrogen Benchmark prestained molecular weight markers, Cat. No. 10748-010
[2] Tween -20 was from Pierce Cat. No.
[3] Obtained from Dr. Ken Platt, College of Veterinary Medicine, Iowa State University

Tween and a 1:250 dilution of monoclonal antibody to PRRSV[4] (SDOW-17). The blots were rinsed and incubated with a 1:1000 dilution of horseradish peroxidase labeled rabbit anti-mouse IgG (heavy and light chain specific)[5] in PBS-Tween on a rocker for 1 hr 37°C. Blots were washed and developed with TMB Membrane Peroxidase Substrate solution[6] according to the manufacturer's instructions.

Enzyme-linked immunosorbent assays and data analysis: ELISA's were done using Nunc[7] flat bottom 96-well plates; for fluorescence experiments Costar[8] black, clear bottom plates were used. The PRRSV protein was used in triplicate wells at concentrations from10 µg/well down to10 pg/well; controls wells contained either no antigen, or contained antigen, but no monoclonal antibody. Monoclonal antibodies from Ken Platt were used at a dilution of 1:50 in BPS-Tween and the monoclonal antibody SDOW17 was used at a dilution of 1:250 (as per direction of the manufacturer). FITC-labeled goat-anti-mouse IgG (H + L chains) was used at a dilution of 1:25 diluted in 10% normal horse serum (manufacturer suggestion is to use a dilution between 1:20 and 1:50; the 1:50 gave no results in a previous experiment). After removing excess conjugate, the plates were washed with PBS-Tween and once with 0.1 M phosphate buffer, pH 7.2. Plates were read using a fluorometer[9] at an excitation wavelength of 485 nm and an emission wavelength of 515 nm (according to the manufacturer's instructions). Each data point was corrected for background fluorescence and the three data points were averaged using the plate reader's software program. The data were plotted using Excel. FITC conjugate was titrated by using a 2-fold dilution series and its fluorescence measured in terms of relative fluorescence units. Data were plotted using Excel.

Tonsil tissue processing.

a. Preparation of tissue. Tissue, approximately 1 g was placed in pre-cooled (liquid nitrogen) mortar, liquid nitrogen was added and the tissue ground to a fine powder, transferred to a 15-mL Corex tube. Four volumes of extraction solution containing 0.32 M sucrose, 20 mM HEPES, pH 7.2, 4 mM phenylmethylsulfonyl fluoride (PMSF) was added. The suspension was stirred and extracted overnight at 5 C. The suspension was centrifuged at 15,000 ×g and the pellet and supernatants were saved, and stored at -20C. Protein was determined with the Bicinchonic Acid Protein Assay kit (BCA) procedure[10] (Sigma), using bovine serum albumin as a standard. Samples were denatured and reduced using the Invitrogen sample buffer and protocols as described above. SDS-PAGE was performed using 10 ug/well as described above using 16% homogeneous gels (Invitrogen). A second gel was used for western blotting to nitrocellulose as described above. The blots were incubated with monoclonal antibody 15E (Dr. Ken Platt, College of Veterinary Medicine, Iowa State University) diluted 1:50 and developed using goat-anti-mouse IgG, horseradish peroxidase conjugate[11] (Jackson Laboratories) and 4-chloronaphthyl-hydrogen peroxide substrate solution (5).

OLED design and procedures: A typical schematic of an OLED-based sensor (6) and a working blue 4,4'-bis(2,2'-diphenylvinyl)-1,1'-biphenyl (DPVBi)-based OLED are shown in Figs. 8 and 9, respectively. For PRRSV detection, the OLED array was fabricated on a glass slide and two pixels of the blue DPVBI were used as the excitation source. A long pass filter (610 nm) was placed in front of the photomultiplier (PMT) detector window to minimize the background light. Fig. 8 shows the "front

[4] Rural Technologies, Brookings, SD Cat. No. SDOW-17
[5] Jackson Immuno Research Laboratories, Cat. No. 315-035-003
[6] Kirkegaard-Perry Laboratories, Cat. No. 50-77-00
[7] Nunc-Nalgene, Cat. No. 475094
[8] Costar, Corning Incorporated, Corning, NY, Cat. No. 3601
[9] Molecular Devices Model Gemini EM
[10] Sigma Chemical Company, Cat. No. BCA-1 and B-9643
[11] Jackson ImmunoResearch. Cat. No. 315-035-003

detection" mode of operation, where the photodetector (PD) is placed in front of the analyte. In the "back detection" mode, the PD is placed behind the OLED array; the PD then monitors the photoluminescence that passes between the gaps of the OLED pixels. The OLED was operated in a pulsed mode, typically using a 100 µs pulse with a frequency of 20 Hz.

Four solutions 100 µL each, 0.1 M PBS, pH 7.2, Ru-IgG of concentrations of 1X, 2.5X and 5X were deposited in the wells of a black Costar plate. For photoluminescence measurements, the corresponding well in the array was aligned on top of 2 OLED working pixels. The gap between the 2 pixels and anywhere else that are enclosed by the well bottom but not covered by the pixels are possible pathway for the luminescence of Ru.

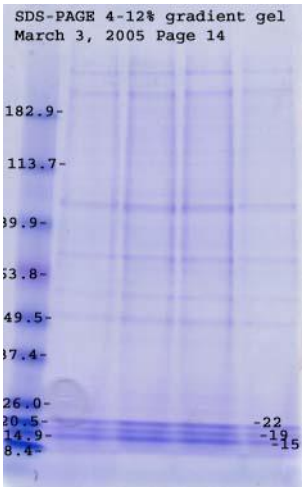
Results and Discussion

Objective 1: Design and construct the photoluminescence-based sensor for detection of PRRSV antigen, nucleic acid, and antibody.

PRRSV protein antigen detection

Before the sensor could be constructed, the viral proteins needed to be analyzed, characterized and quantified. In addition, the binding of the monoclonal antibody SDOW-17 also needed to be evaluated. We used SDS-PAGE for viral protein analysis and purity analysis. A total of 680 µg of viral protein was isolated, dissolved in 1 mL of 0.01 M PBS, pH 7.2, and aliquoted in 100 µL quantities and stored at -89°C. As shown on the 4-12% gradient gel, some high molecular weight proteins were present in the viral preparation. The apparent molecular weights of the proteins as calculated from the gradient gel are 22000, 19,000 and 15,000 for the envelope (E) protein, matrix (M) protein, and the nucleocapsid (N) protein, respectively (Fig. 1A). On the 16% homogeneous gels these proteins have apparent molecular weights of are at 25,000, 19,000 and 15,000 (Fig. 1B) compared to 26,000, 19,000 and 15,000 as reported by Nelson et al. (1993) (7) and Jusa et al. (1997) (2). The difference in molecular weight of the envelope protein could be due to differences in the amount of ammonium persulfate used in SDS-PAGE (7).

A.



B

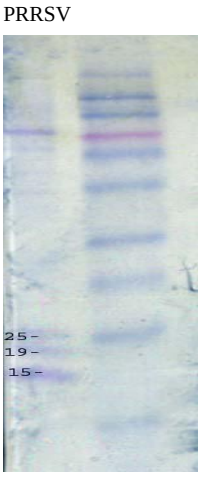


Fig. 1A. SDS-PAGE of purified PRRSV protein in a 4 - 12% gradient gel. PRSSV proteins were denatured and reduced. Each lane contained 10 µg protein. Molecular weight markers of the standards and the Envelope protein (E), Matrix protein (M) and nucleocapsid protein N) are indicated and have apparent molecular weights of 22, 19, and 15 kilodalton.

Fig. 1B. SDS-PAGE of purified PRRSV protein in a 16% homogeneous gel. PRSSV proteins were denatured and reduced. Each lane contained 10 µg protein. Molecular weight markers of the standards are the same as in gel A. The

Envelope protein (E), Matrix protein (M) and nucleocapsid protein N) are indicated and have apparent molecular weights of 25, 19, and 15 kilodalton.

Western blot analysis using the SDOW 17 monoclonal antibody showed no reaction with the viral proteins. This was not expected as Nelson et al (7) reported reaction with the nucleocapsid protein of a different strain of the PRRS virus. Recently, Plagemann (2005) (9) reported that SDOW-17 does not react with PRRSV on a Western blot.

Because of the lack of reactivity of the commonly used SDOW-17 monoclonal antibody, we searched for other monoclonal antibodies to PRRSV. Dr. Ken Platt, College of Veterinary Medicine, Iowa State University, kindly made available to us six monoclonal antibodies prepared to PRRSV nucleocapsid protein. These proteins reacted with all three proteins (Fig 2) and weakly with some higher molecular weight proteins or components. In addition, we did observe a reaction with the SDOW-17 monocolonal antibody. We have no explanation for this. These results will be verified in additional western blotting experiments.

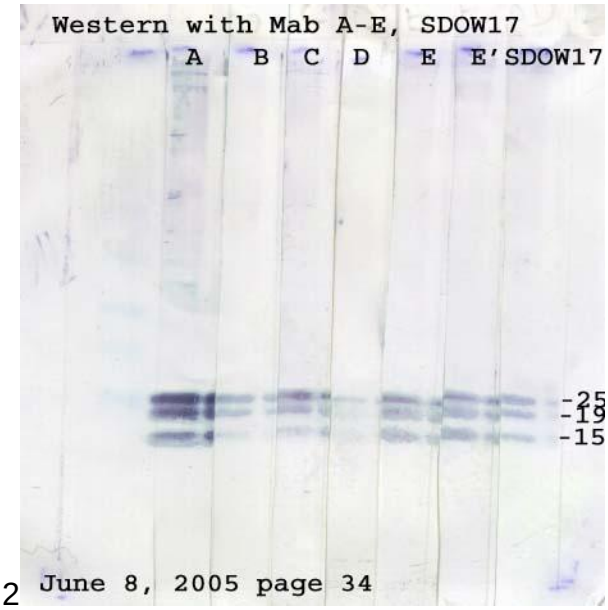


Fig. 2

Fig 3

Fig. 2. Western blot analysis of PRRSV protein. The monoclonal antibodies used are indicated. The apparent molecular weights of the envelope protein (E), the matrix protein (M) , and the nucleocapsid protein (N) are indicated and are 25, 19, and 15 kilodalton, respectively.

Fig. 3. SDS-PAGE of the native PRRSV antigen in a 16% homogeneous gel. The apparent molecular weight of the complex is greater than 182.9 kilodalton.

We also performed electrophoresis under non-denaturing conditions and blotted the native gel. Fig. 3 shows the gel under native conditions. However no transfer of protein occurred as the complex is

probably too large to be transferred from the gel, or alternatively, different electrotransfer procedures need to be employed.

The enzyme immunoassays indicated that a concentration of viral protein antigen of 10 ng/well gave a sufficient fluorescence response for detection using the fluorescein label (Fig. 4). We expect that other goat anti-mouse IgG-labeled fluors, such as Alexa 488 and Alexa 512 will increase sensitivity and should detect lower amounts of viral antigen. In addition these fluors also will provide greater sensitivity when the sensor device will be constructed. Ruthenium fluor will also be used and evaluated. In the latter case, the ruthenium fluor will be conjugated to unlabeled goat anti-mouse IgG.

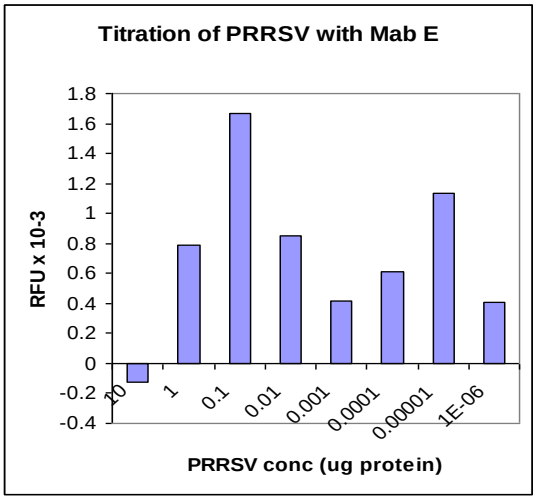


Fig. 4. ELISA results of monoclonal antibody binding to different concentrations of whole PRRSV protein antigen. Monoclonal antibody 15E to nucleocapsid protein was used at a dilution of 1:50. A biphasic curve is observed, which is probably due to the presence of more than one monoclonal antibody with a different specificity and binding constant to one of the PRRSV proteins. Multiple binding activities were also observed in the Western blot (see Fig. 2).

Detection of nucleic acid

No experiments have been initiated to detect nucleic acids. Once the protein antigen detection experiments have been completed, experiment to detect nucleic acids will be initiated. This part of Objective 1 will be continued in a potential new grant application for fall of 2006.

Detection of antibody to PRRSV

Antibody detection experiments were performed by fluorometry, using serum samples from infected pigs, and samples from negative pigs.

These samples were obtained from a larger study funded by the BIGPIG project and were obtained from Dr. Jeff Zimmerman, College of Veterinary Medicine, Iowa State University.

The results from 5 sera, sera from three infected pigs (#104, #105 and #106) and from two PRRSV-negative pigs are shown below in Figures 5 and 6. Sera were titrated by serial two-fold dilution starting with a 1:5 dilution using 10 ng and 20 ng of PRRSV antigen per well, Figures 5 and 6, respectively. At a serum dilution of 1:320 (serial dilution number of 7) all three positive sera were positive and the control sera were negative. However, the control sera would be considered “positive” at a dilutions of 1:5, 1:10, 1:20 and 1:40 (serial dilution number 1, 2, 3, and 4), but “negative” at 1:80 (serial dilution number 5) using the 10 ng/well of PRRSV. The control sera would be considered “positive” at dilutions 1:5, 1:10 and 1:20 (serial dilution number 1, 2, and 3), but

"negative" at 1:40 (dilution number 4) using the 20 ng of PRRSV antigen per well. The positive and negative cut-off ranges were obtained from 16 data points at a dilution of 1:640 (dilution number 8). The negative cutoff was determined as the average of 16 values (A_{405} = 0.049), plus 3 standard deviations (3 ×0.009) = A_{405} = 0.076. In order to get a more reasonable estimate of the negative /positive cut-off value, at least 124 PRRSV-negative sera will be tested. In addition, these ELISA results were conducted on single well two-fold dilution series. Experiments will be repeated with triple well dilution series. Also, based on the titration curves shown below, the data suggest that a two-dilution assay in triplicate at dilutions of 1:100 and 1:200 should distinguish the positive from the negative serum samples.

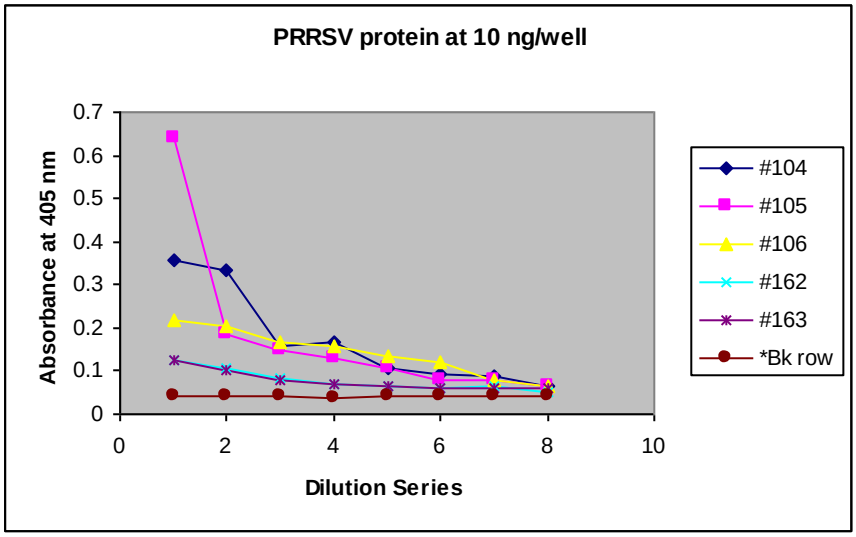


Fig. 5

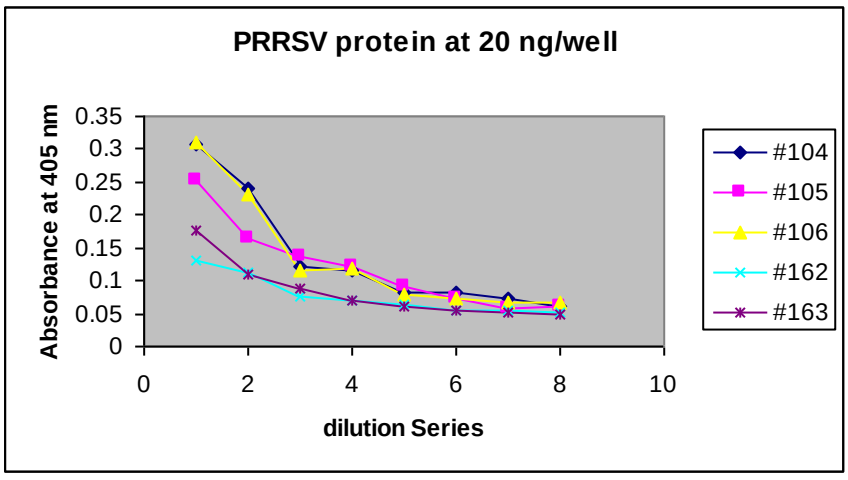


Fig. 6

Figures 5 and 6. Enzyme-linked immunoassay for detection of antibody to PRRSV in pig serum samples. Both 10 ng and 20 ng PRRSV protein antigen/well was used in the ELISA assay using rabbit anti-porcine IgG-horseradish peroxidase conjugate. Positive serum samples, #104, #105, #106; negative serum samples, #162, #163. A single well two-fold dilution series was tested starting at a 1:5 dilution.

<u>Objective 2: Evaluate sensitivity and specificity of the sensor utilizing PRRSV-spiked samples</u>

In the interest of time, we did not spike samples with PRRS virus, but instead used the Ru-anti porcine IgG to optimize the OLED-based detection system (see Fig. 7). Fig 7 depicts the actual photoluminescence signals emitted using OLED excitation. Three different antibody concentrations were used, 1 X, 2.5 X and 5.0X. There was no difference in signal between the 2.5X and 5X antibody concentrations, probably indicating the system was saturated. Schematically, the design of the OLED is shown in Fig. 8 and a working OLED showing a single pixel blue OLED is shown in Fig. 9.

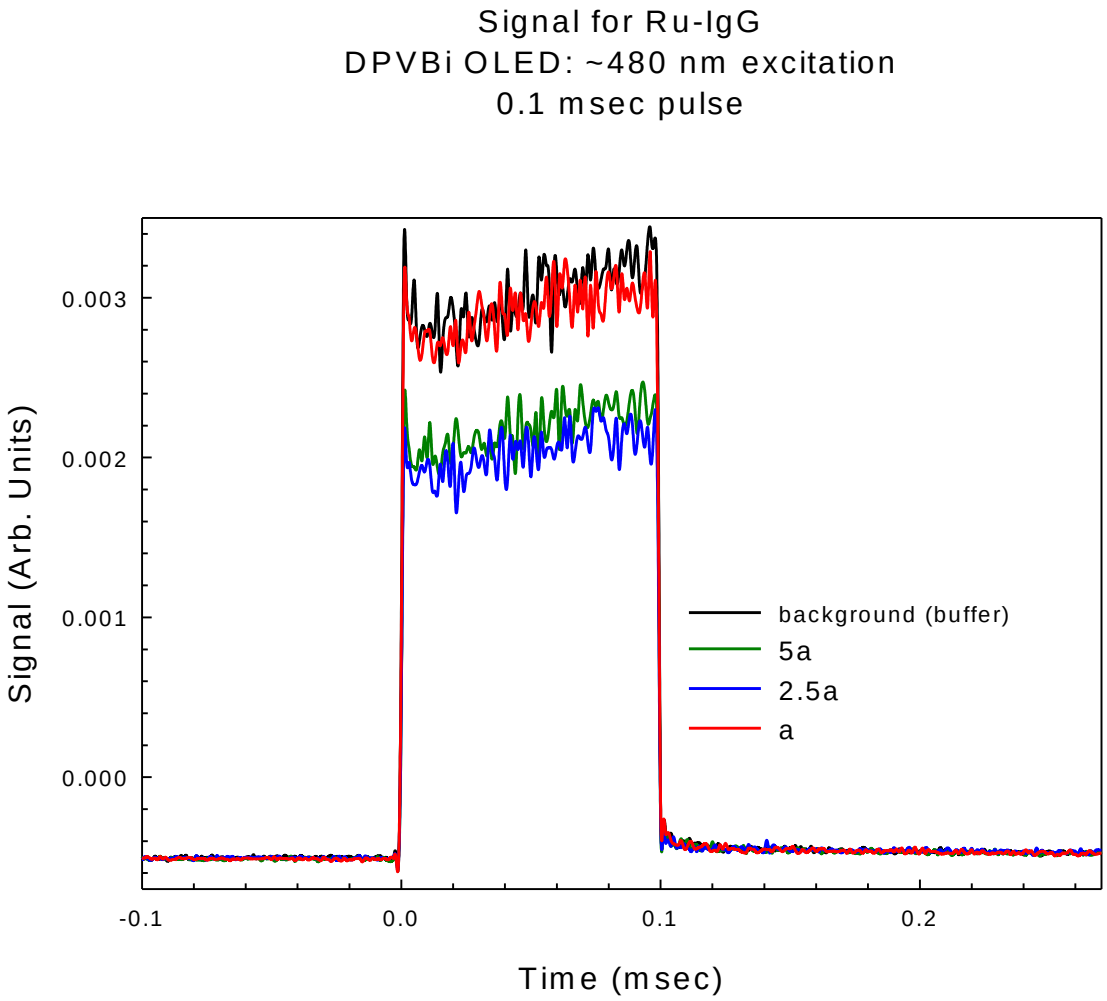


Fig. 7. Actual photoluminescence signals produced by the OLED excitation using different concentrations of R-IgG antibodies; *a, 2.5a, and 5a*, antibody concentrations of 1X, 2.5X and 5X, respectively.

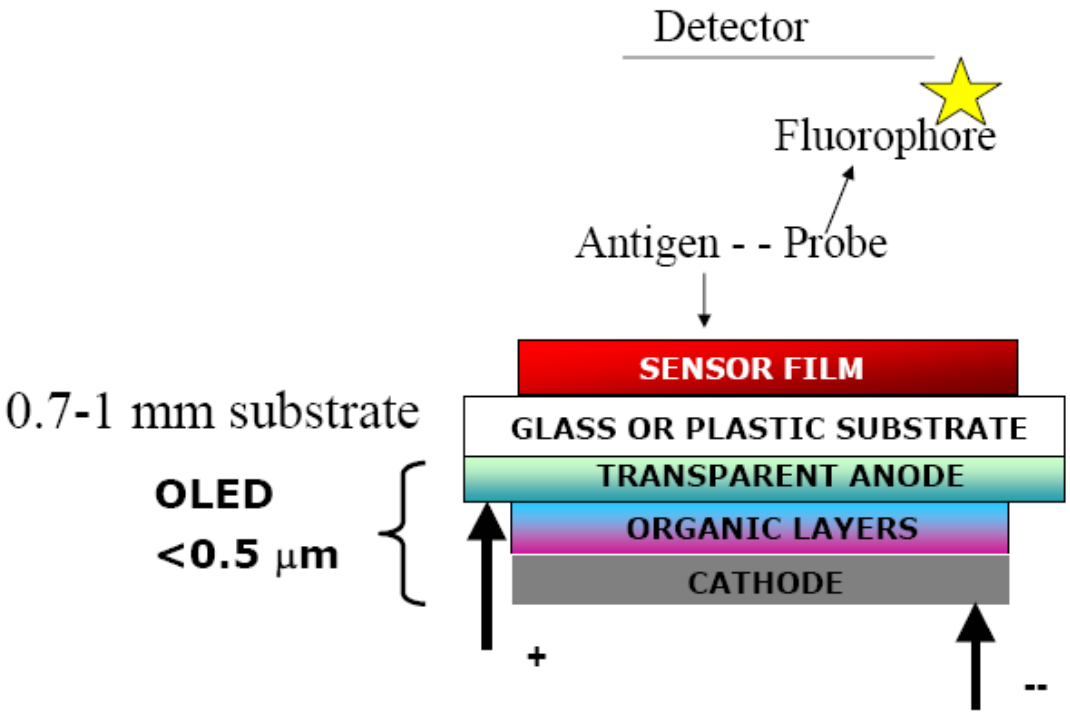


Fig.8 Schematic of an OLED detection system.

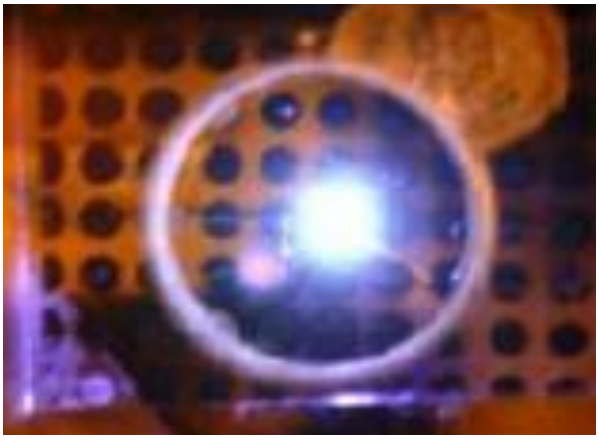


Fig. 9. Example of a blue OLED showing a single pixel emitting light.

Experiments are continuing to optimize conditions for OLED excitation and photoluminescence detection. Based on the results shown in Fig. 7, where the photoluminescence signal reduces in the presence of increasing Ru-IgG concentrations, it appears that in addition to the photoluminescence signal, absorption by the Ru-IgG takes place. Experiments for using and optimizing absorption for PRRSV detection using the OLED system will continue.

Objective 3. Evaluate the sensitivity and specificity of the sensor on tissue samples collected from pigs experimentally infected with PRRSV.

We have tested a limited number of tonsil tissues from infected pigs (#104, #105, #106) and control pigs (#162, #163) (samples supplied by Dr. Jeff Zimmerman, College of Veterinary Medicine, Iowa State University) pigs #104, #105, #106, #162, #163 were the same pigs from which the sera were collected that were tested in the enzyme immunoassay described above.

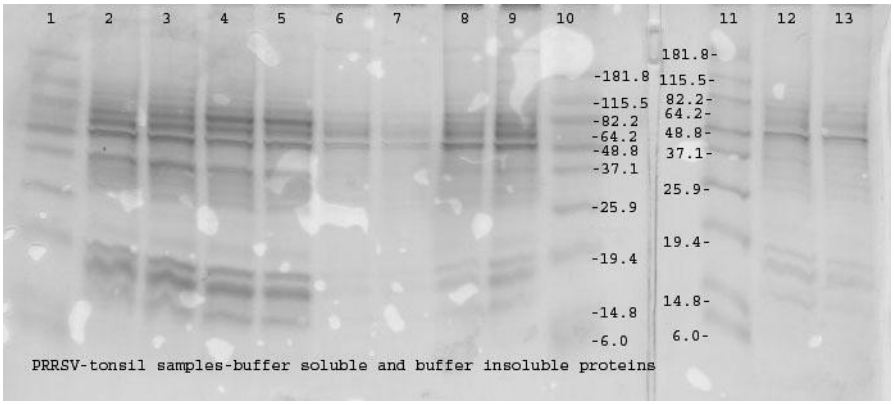
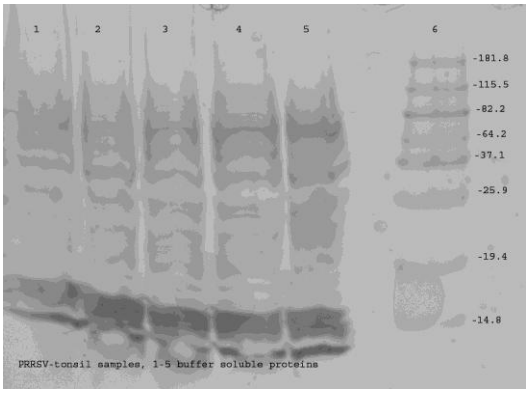


Fig. 10. SDS-PAGE of tonsil tissue supernatant and pellet (buffer soluble and buffer insoluble proteins, respectively. Lanes 1, 10 and 11, molecular weight markers. Lanes 2, 3, 4, 5, and 6, buffer-soluble proteins from tonsil tissues, #104, #15, #106, #162, #163, respectively; Lanes 7, 8, 9, 12, and 13, buffer- insoluble proteins from tonsil tissues #104, #105, #106, #162, #163, respectively.

Fig 10 shows an SDS-PAGE of the tonsil tissues, both of the buffer-soluble fractions and the buffer-insoluble fractions.

Fig. 11 shows a repeat of the gel using the buffer soluble samples and Fig. 12 shows a Western blot of the buffer-soluble fractions using monoclonal antibody 15E to detect the PRRSV antigen.

Fig. 11 Fig. 12



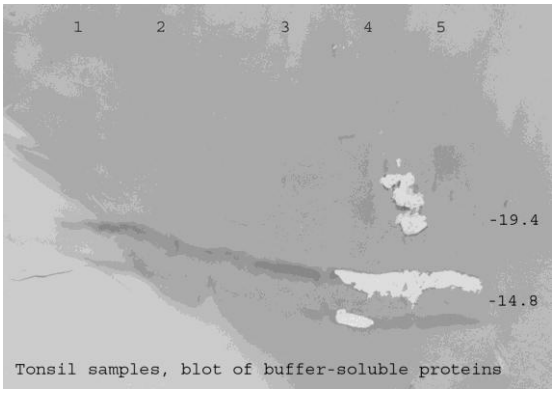


Fig. 11, SDS-PAGE of buffer-soluble proteins. Lanes 1-5, Samples #104, #105, #106, #162 and #163. Lane 6, molecular weight markers in kilodalton.

Fig. 12, Western blot of samples shown in Fig. 11. Lanes 1-5, Samples #104, #105, #106, #162, and #163. Blot was incubated with Mab 15E and visualized with horseradish peroxidase-conjugated goat-anti mouse IgG and developed with TMB substrate solution. Numbers to the right indicate molecular weight markers

The western blots with these tissue samples show a dark band at approximately 15 kDa (Fig. 12). The blank spots for lanes 4 and 5 indicate that the antibody-conjugate complex washed off the blot. It is curious that the positive as well as the control samples showed a reaction with the monoclonal antibody. The reason for this is not clear. To clarify this result, more samples need to be run and the reactivity of the monoclonal antibody with these proteins needs to be further investigated.

IV. Conclusions and further work

1. PRRSV strain NADC-8 was isolated, quantified and characterized. This viral preparation may contain additional cell proteins. Future preps will include purification of the nucleocapsid protein itself, or purchase/obtain the purified recombinant protein from ATG Labs[12].

2. Initial Western blots experiments showed no reaction with the SDOW-17 monoclonal antibody, confirming a recently published report by Plagemann (9). Subsequent Western blot experiments using a different buffer system and gel system from Invitrogen showed reactivity with the SDOW-17 monoclonal antibody. We will further explore this discrepancy.

3. ELISA performed with monoclonal antibody 15E (from Ken Platt) showed that a 50% of maximal RFU was obtained with 10 ng/well of PRRS viral protein antigen.

4. ELISA with porcine sera from infected and non-infected pigs showed that the ELISA test can distinguish PRRSV positive from PRRSV-negative sera.

5. Western blots with tonsil tissues were inconclusive and will be repeated.

6. OLEDS were constructed and tested with the Ru-anti-porcine conjugate. Experiments to optimize conditions for OLED photoluminescence output are on-going. Ru-IgG conjugate also exhibits an intense absorbance at 480 nm. This is being explored for use with the OLED with an absorption detection mode.

[12] ATG Labs, Eden Prairie, MN as reported in the June 2005 PRRSV Newsletter edited by Tahmi Perzichilli.

References

1. Kim, HS, Kwang J, Yoon IJ, Frey ML, Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. Arch virol 133, 277-483 (1993).
2. Jusa ER, Inaba Y, Kounu M, Asagoe T, Uwatoko K, Yamaura K, Hirose O, characterization of porcine reproductive and respiratory syndrome virus hemagglutinin, J Vet Med Sci 59, 281-286 (1997).
3. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265-275 (1951).
4. Laemmli UK, Cleavage of structural proteins during assembly of the bacteriophage T4. Nature (London) 227,680-685 (1970).
5. Towbin H, Staehelin T, Gordon J, Electrophoretic transfer of proteins from acrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA, 76, 4350-4354 (1979).
6. Shinar, R, Choudhury, B, Zhou, Z, Wu, H.-S, Tabatabai, LB, Shinar, J. Structurally integrated organic-light emitting device-based sensors for oxygen, glucose, hydrazine and anthrax. Proceedings of the International Society for Optical engineering, 5588: 59-69 (2004).
7. Nelson EA, Christopher-Jennings J, Drew T, Wensvoort G, Collins JE, Benfield DA, Differentiation of U.S. and European isolates of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. J Clin Microbiol 31, 3184-3189 (1993).
8. Lobos SR, Mora GC, Alteration in the Electrophoretic mobility of OmpC to variations in ammonium persulfate concentration in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis, 12, 448-450 (1991).
9 Plagemann PGW, Epitope specificity of monoclonal antibodies to the N-protein of porcine reproductive and respiratory syndrome virus determined by ELISA with synthetic peptides. Vet Immunol Immunopathol 104, 59-68 (2005).

Lay Interpretation

This report describes results obtained for the optimization of reagents to be used for the construction of the novel photoluminescence sensor device. PRRSV strain NADC-8 was purified and characterized by gel electrophoresis, Western blotting and ELISA. We found initially that the widely used monoclonal antibody for detecting PRRSV did not react in the Western blot format. This result is in agreement with a recently published paper by Dr. Plagemann from the University of Minnesota. This prompted us to search for different monoclonal antibodies for evaluation. Six monoclonal antibodies to the nucleocapsid protein of PRRSV were kindly provided by Dr. Ken Platt from the College of Veterinary Medicine, Iowa State University. All six monoclonal antibodies reacted with the PRRSV antigen in the Western blot format as well as in the ELISA format. To improve sensitivity of detection, we used a fluorescent labeled reagent to detect monoclonal antibody binding to the PRRSV nucleocapsid protein antigen. Newer fluorescent labels such as ruthenium have been evaluated for use with the OLED–based and fluorometry detection systems for the detection of for PRRSV. However, unanticipated problems were encountered using the new laser dye with the OLED sensor. Experiments to further optimize the OLED detection system for the detection of PRRSV protein antigens are on-going. An ELISA was developed using PRRSV protein antigens that distinguished sero-positive from sero-negative serum samples. The data suggest that a two-dilution endpoint ELISA could be used to detect PRRSV in serum samples. For more information please contact Louisa B. Tabatabai at ltabatab@nadc.ars.usda.gov.