

# Diagnostic INSIGHTS



Volume 1, Number 1

January/February 2007



Gary Anderson, DVM, MS, PhD  
Director of Diagnostic Laboratory

We are pleased to initiate our newsletter, *Diagnostic Insights* and welcome you to this inaugural issue. Our objective is to use *Diagnostic Insights* as one of the ways to emphasize our commitment to **partnering** with you, our key stakeholders and colleagues in practice. We will be very intentional in our goal of providing relevant, timely, and practical information to you. Diagnostic tips, disease alerts/issues, new tests, value-added services, and access to laboratory and college personnel will be included in each issue of *Diagnostic Insights*.

Numerous changes and many other positive, exciting things are occurring in the Kansas State Veterinary Diagnostic Laboratory (KSVDL). Many of you took time from your busy schedules to assist us by answering a third-party survey this past year and we have made every effort to listen. Improvements and actions taken throughout the KSVDL are ongoing and in direct response to the feedback we received from you during that survey. We want to continue to hear from you because it is necessary to have open, honest communication if we are going to achieve our goal of becoming your First-Choice diagnostic laboratory – we believe it is all about effective communication and **partnering**. Everything KSVDL is doing is driven by the

desire to fulfill our mission quality service. That service must be accurate, timely, relevant, and value-added.....we (and so should you) expect nothing less!

For the first issue of *Diagnostic Insights*, Molecular Diagnostics has been targeted as an area where we can most effectively enhance our services to you in the short term. We hope that you enjoy hearing about the benefits of the technology and new tests offered in Molecular Diagnostics.

## *KSVDL Takes a Leadership Role in Molecular Diagnostics*

by Dick Oberst, DVM, PhD,  
Director of Molecular Diagnostics

Molecular diagnostics or the ability to detect specific bacterial, viral, or parasitic nucleic acid (i.e., DNA or RNA) is one of the fastest growing technologies in biological sciences. Most often molecular diagnostics involves Polymerase Chain Reaction (PCR) technologies and automated PCR systems, frequently called real-time PCR systems.

The Molecular Diagnostic Section is the newest section in the KSVDL and our goal is to offer our clients a wide variety of molecular diagnostic tests and to respond rapidly to those requests by completing those services in a timely manner. We are also currently involved in reorganizing and remodeling our labs to further enhance our molecular diagnostic capabilities and capacity to better serve those needs.

A recent example of the increasing importance that molecular diagnostics is playing in the KSVDL is our involvement in the National Animal Health Laboratory Network (NAHLN). A major focus of NAHLN is to develop the infrastructure that allows facilities the ability to rapidly perform standardized real-time PCR assays as screening tests to rapidly detect high risk and economically important foreign animal disease (FAD) agents, and then to network with other Federal and State facilities to initiate emergency responses measures. In 2006 personnel from the KSVDL have undergone NAHLN proficiency training/testing to be qualified to perform real-time PCR assays to detect Foot & Mouth Disease Virus, Classical Swine Fever Virus, Avian Influenza, and Exotic Newcastle Disease Virus.



These KSVDL personnel participated in the NAHLN proficiency training/testing to be qualified to perform real-time PCR assays to detect Foot & Mouth Disease Virus and Classical Swine Fever Virus.  
l to r, Mike Hays, Joe Anderson, Dr. Dick Hesse, Jessica Jewell, Dr. Su-Ann Murdock, Heather Wisdom, Dr. Dick Oberst.

*“Veterinary practitioners will benefit from our involvement with NAHLN in several ways, states Dr. Oberst. First from the bigger picture of the protection against foreign animal disease risks and from KSVDL being able to offer unique testing capabilities which are economical and timely for the day-to-day practice requirements”.*



## Expanded PCR Capabilities for Improved Bacterial Diagnostics

by Muthu Chengappa, MS, Research Assistant

Molecular diagnostic testing of infectious bacterial agents using Polymerase Chain Reaction (PCR) has become a valuable tool for diagnosing many diseases. The sensitivity and specificity of organism detection at any stage during the course of an infection, even when no clinical signs of disease are present, makes PCR an exquisite monitoring technique. Compared to serological testing, PCR-based testing offers reduced cross-reactivity, fewer false positives, and detection independent of serum antibody levels. PCR-based testing also yields more rapid diagnosis of slow-growing or difficult-to-isolate agents, such as *Bordetella* species, *Hemophilus parasuis*, *Mycoplasma* species and many more, than can be obtained by traditional culture techniques.

The KSVDL now offers several new PCR-based diagnostic assays for clinical samples (nasal/tracheal swabs, tissues,

guttural pouch washes, etc). The new tests are for *Bordetella bronchiseptica*, *Bordetella avium*, and four *Streptococcus* species: *canis*, *suis*, *bovis*, and *equi*. We can also differentiate *S. equi* subspecies *equi* and *S. equi* subspecies *zooepidemicus*. Additionally, using RAPD-PCR we are able to differentiate *Actinobacillus pleuropneumoniae* serotypes 1 through 15 and *Moraxella bovis*.

Our research and observations lead us to conclude that *Bordetella bronchiseptica* may be under-diagnosed because it grows slowly in culture. Using PCR, it can be detected in nasal and tracheal swabs more accurately and faster.

We continue to offer the popular PCR assays for the detection of *Actinobacillus pleuropneumoniae* and *Hemophilus parasuis*.



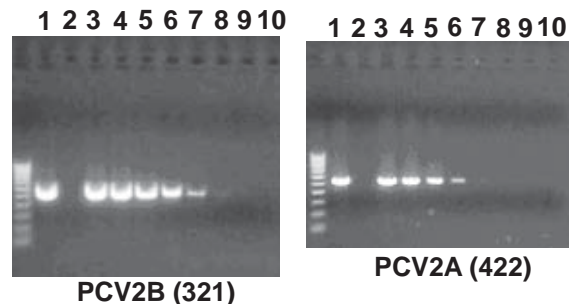
## PCVAD Differential Diagnosis via PCR

by Richard Hesse, PhD, Director of Diagnostic Virology

Porcine circovirus associated disease (PCVAD) is becoming the scourge of the swine industry. PCVAD presents as an acute severe illness that affects finish hogs. Mortality rates in affected units are typically 15-20% but can be as high as 70%.

Infected animals become rough and fade away—often dying within a week of exhibiting clinical signs. Porcine circovirus 2 (PCV2) and an infectious co-factor, usually Porcine Reproductive & Respiratory Syndrome (PRRS), *Mycoplasma hyopneumoniae*, swine influenza virus or other agents are typically identified concurrently at the diagnostic laboratory. The PCV2B genotype (Restriction Fragment Length Polymorphism [RFLP] type 321) of PCV2 is strongly associated with severe PCVAD whereas animals infected with the PCV2A genotype (RFLP type 422) tend to have mild or no disease.

**Differential PCR Test Research:** A unique differential polymerase chain reaction (PCR) test that identifies the PCV2A and PCV2B genotypes of circovirus has recently been developed in Dr. Rowland's laboratory, Diagnostic Medicine /Pathobiology (DMP) at Kansas State University. This differential PCR test is now being implemented by KSVDL and used to rapidly identify which circovirus genotype is present and to determine the "viral load" in that animal. The differential test is being used to monitor pig flows to ensure that PCV2B infected animals do not enter a "clean" flow. The test is also used to determine viral loads in serum or tissue homogenates to help understand the basic pathogenesis of the infection and how other agents contribute to the severity of the disease. The PCR technology is essential in determining if circovirus is truly causing the problems observed in the herd, which will be important for establishing control and prevention protocols.



PCV2 Differential PCR being used to determine the viral load of both genotypes present in a diseased pig. Sequential dilutions demonstrate "loads" of  $> 10^6$  for PCV2B and  $10^5$  for PCV2A.

## KSVDL Welcomes Dr. Hesse

Dr. Dick Hesse is the new Director of Diagnostic Virology at the Kansas State Veterinary Diagnostic Laboratory. He received his Master of Science (Biology) from South Dakota State University and his Doctor of Philosophy (Virology) from the University of Nebraska. Dr. Hesse has authored or co-authored more than 40 publications, presentations, and/or patents. In addition, he has led the development of at least 12 USDA-licensed vaccines. Dr. Hesse's research has included infectious disease pathogenesis and vaccine development, with his most recent focus on PRRS (porcine respiratory and reproductive syndrome) and PCVAD (porcine circovirus-associated disease). We are pleased to welcome Dr. Hesse to Kansas State University, DMP and the KSVDL. His wealth of knowledge and expertise will benefit KSVDL clients

-Gary Anderson, Director of KSVDL



# Accurate and Rapid BVD Testing for Persistent Infection

by Cindy Chard-Bergstrom, Microbiologist III, HT (ASCP) QIHC

**B**ovine viral diarrhoea (BVD) virus can lurk unnoticed in the form of persistently infected animals in your clients' herds resulting in major economic losses each year. For this reason it is important to routinely identify and remove infected animals.

The KSVDL presently offers two different tests for animals persistently infected (PI) with bovine viral diarrhoea virus using an ear notch or skin sample. These two tests are the BVD antigen capture ELISA (BVD-ACE) and the BVD immunohistochemistry test (BVD-IHC). ACE provides faster turn around and IHC is still considered the "gold standard" for identifying PI animals. Both tests require a skin sample approximately 1cm by 1cm. Samples for both tests should be submitted with paperwork indicating the tube number marked on the tube and the corresponding animal ID to ensure precise individual animal results. Pre-filled sample submission containers are available for both tests at a cost of \$0.30 each, or the formulas for Phosphate Buffered Saline (PBS) or 10% Neutral Buffered Formalin (NBF) may be obtained from the laboratory by calling toll free **1-866-512-5650**.

**BVD-ACE SAMPLING:** BVD-ACE testing requires the sample to be placed in 2 mL of (PBS) immediately upon collection. Samples should be completely submerged in the PBS because dried out samples may yield false negative results.

Samples in PBS should be refrigerated as soon as possible after collection and samples should be delivered to the laboratory within 2 days of collection. Samples that cannot be delivered within 2 days should be frozen and kept frozen until delivered to the lab. Samples are tested and any reactors are rechecked to verify positive or negative status. The test takes a minimum of 5.5 hours to run. Turnaround time for this test is 1 to 2 working days.

**BVD-IHC SAMPLING:** BVD-IHC test requires samples to be placed in (NBF) immediately after collection. Samples should be submitted to the laboratory within 5 days after collection. Turnaround time for this test is usually 3 to 4 working days.



l to r Shelly Christenson, Nathan Rodriguez, Shon Koenig, Dr. Ida Piperisova and Cindy Chard-Bergstrom. Not pictured, Micheal Lesser

## Continuing Education Opportunities at K-State, Spring 2007

by Linda M. Johnson, Ph.D.

### KSVDL Presentations

**March 3: Veterinary Technicians Conference**

**June 3-6: 69<sup>th</sup> Annual Conference for Veterinarians**

Both of these conferences will include a session facilitated by Jeff Anderson that will let participants experience the operations of the KSVDL as well as learn how to access KSVDL from their clinic. Participants will use a computer to learn how KSVDL will benefit clinics for submission of lab samples and retrieval of test results. Each participant will learn about the Laboratory Information Management System (LIMS), enter clinic data into the computer, receive results, and have the option to setup KSVDL web access for their clinic.



Jeff Anderson, BS, accessing veterinary practices to KSVDL

#### Dates to Remember:

*Canine Care Workshop for Pet Breeders and Veterinarians*, Jan 27

*Emergency Medicine Conference* - Feb 3

*Equine Reproduction for Veterinarians* - Feb 10 and 11

*Small Animal Medicine Conference on Dentistry* - Feb 11

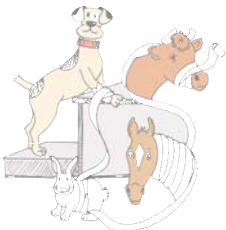
*Equine Reproduction for Horse Breeders* - Feb 17 and 18

*Veterinary Technicians Conference* - March 3

*Frank W. Jordan Seminar: Zoonoses and Pets of Immunocompromised Clients* - March 11

*Bovine Conference on Health and Production*, April 20 and 21

*Annual Conference for Veterinarians* - June 3-6 - 100 years of graduating veterinary students!



**Capture CE credits in your clinic! VetBytes, on-line for two weeks!**

March 5-16, Outpatient Pain Management

April 9-20, Bovine Spongiform Encephalopathy

#### More Information?

[www.vet.k-state.edu](http://www.vet.k-state.edu) Click on Continuing Education or call 785-532-5696, ask for Linda or Marci





The mission of the Kansas State Veterinary Diagnostic Laboratory (KSVDL) is to assist clients in the detection, prevention and understanding of diseases. The KSVDL is a full-service, AAVLD-accredited laboratory, offering a complete range of diagnostic services for all species. The KSVDL strives to provide high quality diagnostic and consultative services to the veterinary profession and animal owners.

**Contact Information:**

**Kansas State Veterinary Diagnostic Laboratory**  
1800 Denison Avenue  
Kansas State University  
Manhattan, KS 66506  
Phone: 785-532-5650  
Toll Free: 866-512-5650  
Fax: 785-532-4481  
Email: [DLabOffice@vet.k-state.edu](mailto:DLabOffice@vet.k-state.edu)

**Rabies: 785-532-4483**  
**Accounting Office: 785-532-4533.**



For information on connecting to WebAccess for real-time test results, contact the KSVDL IT department at 785-532-4682.

Visit our website at:

Web: <http://www.vet.k-state.edu/depts/dmp/service/index.htm>

*Diagnostic Insights* welcomes your suggestions for future articles or comments about current articles.

Send your ideas to Barbara Barkdoll at [bbarkdol@vet.k-state.edu](mailto:bbarkdol@vet.k-state.edu).

**Kansas State Veterinary Diagnostic Laboratory**  
1800 Denison Avenue  
Kansas State University  
Manhattan, KS 66506

