Actinobacillus pleuropneumoniae detection by PCR and strain characterization by genotyping

Fact sheet

APP-PCR

A newly standardized and validated PCR test for detection of Actinobacillus pleuropneumoniae in clinical samples is now available at the University of Minnesota Veterinary Diagnostic Laboratory. This test can detect a minimum of $8.7 \times 10^1$ CFU/ml (Fig. 1 A) and was shown to be specific for detection of A. pleuropneumoniae when tested using DNA extracted from the following swine pathogens: Actinobacillus equuli, Actinobacillus suis, Actinobacillus rossii, Actinobacillus porcinus, Actinobacillus minor, Actinobacillus indolicus, Bordetella bronchiseptica, Erysipelothrix rhusiopathiae, Escherichia coli, Pasteurella multocida, Salmonella sp., Streptococcus suis, and Haemophilus parasuis.

**Figure 1.** Sensitivity of the newly standardized Actinobacillus pleuropneumoniae PCR using 10-fold dilutions of a pure culture (Actinobacillus pleuropneumoniae strain ATCC 33565). M – Molecular weight marker.

APP-PCR was positive when tested using DNA extracted from A. pleuropneumoniae reference strains ATCC 33378, ATCC 33337, ATCC 27088, ATCC 27090, ATCC 27089, and field isolates. PCR was also positive when lungs from naturally infected pigs were tested (Fig. 2). PCR was negative when pathogens other than A. pleuropneumoniae were isolated from the lung, such as Actinobacillus suis, Pasteurella multocida and Streptococcus suis, or when isolation for A. pleuropneumoniae was negative (no growth).

**Figure 2.** APP-PCR results for lung tissues from 3 pigs diagnosed with Actinobacillus pleuropneumoniae pneumonia based on histopathology and bacterial isolation. M – Molecular weight marker.

The new APP-PCR can be used as an alternative tool to diagnose A. pleuropneumoniae infections, especially when bacterial isolation is negative. Negative isolation results may be the result of poor sample handling prior to submission or previous antibiotic treatments. A new A. pleuropneumoniae screening ELISA test offered by the University of Minnesota Veterinary Diagnostic Laboratory can be used in addition to the PCR test to diagnose A. pleuropneumoniae subclinical infections.

**Samples to be submitted for testing (refrigerated):** Lung, whole tonsil, tonsil swabs.

**Expected turnaround time:** 2-10 days. Samples are tested on Monday and results are reported on Tuesday.

SYS.WEBDOC.015 Rev 1 6/29/2012
**Actinobacillus pleuropneumoniae genotyping**

The University of Minnesota Veterinary Diagnostic Laboratory is now offering routine genotyping and genetic analysis of *A. pleuropneumoniae* isolates. Isolates recovered from clinical cases are genotyped and a computer-based analysis is performed to create a dendrogram (Fig 3). The dendrogram contains not only the genomic fingerprint of each isolate, but also detailed clinical information such as date of isolation, herd and site identification, age of affected animals, organs from which the bacterial isolates were recovered, lesions associated with isolation, and antibiotic resistance profiles.

The dendrogram can be used to evaluate the genetic variability of *A. pleuropneumoniae* isolates from a specific herd. It can also be used for selection of strains to be used in autogenous vaccines. A database is created for each herd, and isolates from new clinical cases can be genotyped and compared to previous isolates and to vaccine strains.

![Dendrogram of genotyped Actinobacillus pleuropneumoniae isolates](image)

**Figure 3.** Genetic analysis of *Actinobacillus pleuropneumoniae* isolates recovered from naturally infected pigs. Six different strains were identified by genotyping. The genotyping technique was able to differentiate *A. pleuropneumoniae* strains from the same serotype group (serotype 5).

**Sample submission:**

1. *Actinobacillus pleuropneumoniae* isolates are necessary for genotype testing.
2. Swine tissues (lungs, whole tonsils) or tonsil swabs can be submitted for bacterial isolation and genotyping.
3. *Actinobacillus pleuropneumoniae* isolates can also be forwarded to the University of Minnesota Veterinary Diagnostic Laboratory for genotyping only.

If you have any questions about these procedures, or proper submission of samples, please contact Dr. Kurt Rossow or Dr. Jim Collins at 1-800-605-8787 or by Email at vdl@umn.edu.