

## SWINE HEALTH

**Title:** Evaluation of diagnostic assays for *Mycoplasma hyopneumoniae*: Their potential role in eradication success – npb #05-006

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### Abstract

*Mycoplasma hyopneumoniae* (Mhyo) is an important pathogen in the swine population worldwide. Diagnosis of infection with this organism on an individual pig and herd basis continues to be challenging due to its slow spread within a herd, delayed seroconversion after infection, and genetic differences between isolates. All of these factors play important roles in the often difficult process of accurately detecting Mhyo, especially for eradication purposes. To evaluate the sensitivity and specificity of ELISA and PCR assays and also sampling sites for PCR, a time-course study was performed with groups of pigs challenged with field isolates of Mhyo, *M. flocculare*, *M. hyorhinitis* or *M. hyosynoviae*. Forty-eight pigs were divided into eight challenge groups. Four groups received different Mhyo isolates, two received different *M. flocculare* isolates, and one group each received *M. hyorhinitis* or *M. hyosynoviae*. Two pigs were necropsied at each of the following time points: 28, 70 and 95 days post challenge (DPC). Serum and nasal swabs were collected throughout the study and bronchoalveolar lavage fluid (BAL) and bronchial swabs were collected at necropsy. Three different ELISA assays, DAKO, IDEXX and Tween 20, were performed and all were found to be 100% specific for Mhyo. The DAKO ELISA appeared to be the most sensitive, detecting pigs earlier (14 DPC) and more consistently than either the Tween 20 or IDEXX ELISAs, which did not detect any positives until the 28 DPC time point. The Tween 20 and IDEXX ELISAs overall performed similarly to each other in this study, but differed 24% of the time on individual results from 28-95 DPC. In addition, the Tween 20 and IDEXX ELISAs appeared to detect lower levels of antibodies from one of the Mhyo isolates compared to the other 3 Mhyo isolates. Real-time PCR was run on the nasal and bronchial swabs and BAL collected. BAL was found to be the most reliable sample for detection of Mhyo.

Additionally, a panel of isolates of Mhyo, *M. flocculare*, *M. hyorhinitis* and *M. hyosynoviae* were compiled and tested against a number of mycoplasma-specific PCR assays. All Mhyo PCR assays tested were 100% specific, but several assays showed decreased sensitivity and did not detect all field isolates of Mhyo. A new real-time PCR assay was developed that appears specific and detects 100% of the Mhyo isolates in our collection. In summary, detection of infected animals on a herd basis by serology or PCR early in *M. hyop* infection is unpredictable and results should be interpreted with care. It may be beneficial to perform a combination of the DAKO assay with either the Tween 20 or IDEXX assays to increase diagnostic sensitivity, especially early in infection. Sampling site and PCR assay selection also have a significant impact on test outcome.

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