# College of Veterinary Medicine STATE UNIVERSITY



DETECTING

# Mycoplasma hyopneumoniae Tracheal Sampling

When trying to diagnose Mycoplasma hyopneumoniae infection in live pigs, it is critical to sample MHP colonization sites characterized by respiratory type epithelium, such as the trachea. We have developed some "Best Practice" guidelines to help with getting these samples to the lab. These guidelines will ensure that samples arrive in a manner that is appropriate for processing and testing.

Tracheal sampling can be achieved with a bit of technique and a few tools (snare, oral speculum, laryngoscope, post-cervical Al rod, scissors, gloves, 5ml snap cap tube and 2 mls of PBS).

At the ISU VDL, a variety of MHP testing is available (PCR, ELISA, IHC) Monday - Friday.

Additionally, due to a growing interest in screening expected negative MHP breeding stock by PCR prior to movement and/or as part of a follow-up to MHP ELISA positive test results in expected MHP negative herds, MHP PCR testing through the VDL's Health Assurance Testing Services (HATS) lab is an additional service that provides benefits of additional segregation within the lab and helps the VDL better understand the context of the particular submission.

TRACHEAL

SAMPLING

A short demonstration video is available on the ISU VDL website

vetmed.iastate.edu/vdl

HOW TO:

The basic instructions are as follows:



Safely snare the pig.



Place the oral speculum inside the oral cavity to open the mouth.



Use the laryngoscope to lower the tongue and improve visibility.



Locate the larynx at the back of the mouth.



Gently insert the end of the rod (use the end with the clear plastic adapter). When you enter the trachea you will notice a change in vocalization.



Gently insert the rod up and down the trachea, then quickly remove.



Remove speculum and snare.



Insert the end of the rod into a 5 ml snap cap tube filled with 2mls PBS and cut off rod so that it fits inside the closed tube.



Label the tube and submit to lab with appropriately completed paperwork.

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# **Veterinary Diagnostic** Laboratory

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# Wendy Stensland Receiving

Wendy was born and raised in lowa, except for a few brief years spent in Missouri. She spent most of her youth on her family farm raising hogs. She attended lowa State University, receiving her Bachelors of Science degree in Biology.

Wendy began working in the ISU VDL in 2001. Wendy serves as the mailroom supervisor, providing daily leadership and oversight to the samples and cases received. She also actively manages the program, non-program, and zoonotic disease surveillance and reporting responsibilities at the ISU VDL. In such, she has frequent correspondence and provides the necessary diagnostic and/or disease incidence information to ISU VDL clientele and the appropriate university, state, and federal animal health and public health officials and agencies.

Wendy, along with her husband Scott, have four adult children. She enjoys lifting weights, drinking craft brews, gardening and being outside.

# **Brucella testing:** What's the Best Test?

David Baum DVM, Katie Woodard DVM, Jeff Zimmerman DVM, Luis Gimenez-Lirola PhD, Sheila Heinen BS.

Thrusfield, M., Diagnostic testing, in Veterinary Epidemiology. 1995, Blackwell Science. p. 280.

Sifford, R., Procedures for Handling Swine Herds with Brucellosis or Pseudorabies, USDA-APHIS-VS, Editor. 2018.

Please visit the ISU VDL website for entire reference list

The subject of "Best Test" remains popular among all who breed dogs or move animals across State or international borders. As long as there are options available (https://vetmed.iastate.edu/vdl/diagnostic-tests/), the question will always remain. One thing for you to remember: Brucella testing is a serial testing process. A serial testing process starts with a screening test followed by a confirmatory test of all non-negative samples. Recall, the purpose of a screening test is to detect ANY potential positive animal.

This means there WILL BE nonspecific reactors, which require a confirmatory test. The confirmatory test is part of serial testing and interpretation. Serial testing improves diagnostic specificity and positive predictive value of a test *process* and asks the animal to "prove" it is affected by the condition being tested (1).

# Moving animals across State borders.

First, find out which test, if any, is required for entry of the species-type into the State of destination. If only a negative Brucella test result is required, we recommend the Brucella abortus/ suis Buffered Acidified Plate Antigen (BAPA) Screen, offered each day of the week. This test, while relatively more sensitive than other tests, is lacking in specificity and are prone to subjectivity and variation between individual technicians. We promptly confirm non-negative samples with the Brucella Fluorescence Polarization Assay (FPA) with its results available by the end of the day. The test is simple to perform, rapid, highly reproducible across laboratories and instruments, and reduces the human error and variability that occurs when reading agglutination tests such as the card test. A negative FPA result is the official status (2) of the sample for the species listed in Table 1. Results are available by day's end.

Table 1. Summary of B. abortus/suis BAPA Screen Test.

Test Name	B. abortus/suis BAPA Screen	Brucella FPA
Species	Bovine, Canine, Caprine, Cervidae, Equine, Ovine, Porcine, Ungulates	Bovine, Porcine
Days Tested	MTWTF	MTWTF
Purpose of Test	Screen	Confirmatory

In the event the confirmatory Brucella FPA result is non-negative, the ISU VDL forwards the sample to National Veterinary Services Laboratory (NVSL) for confirmatory testing. NVSL sends their test results to the areaveterinarian-in-charge, the USDA Brucella epidemiologist, veterinarian of the State of origin, and to the ISU VDL. ISU VDL reports only the result of the NVSL confirmatory tests. The Brucella epidemiologist issues the official interpretation of the results.

### Brucella canis.

This is a significant reproductive disease agent of dogs, an intracellular bacterium and often found in breeding kennels throughout the United States (3). While generally thought of as an organism that produces abortions, the clinical signs of B. canis infection are varied and can be misinterpreted (3). Thus, it is possible for infected animals to raise infected puppies that can enter consumer markets (3). Routine whole-kennel screening helps kennel owners and veterinarians detect antibody against, and therefore infection from, B. canis. Any one of several tests detect B. canis antibodies. The essence of diagnosis is time, not in a test result alone (4). Time and retesting become the diagnostician's allies. The ISU VDL offers one test platform for B. canis antibody testing: B.

canis rapid slide agglutination test (RSAT). The RSAT a screening test, designed to detect any likely positive samples. The test has a high rate of false positives, so dogs which test positive are tested with 2-mercaptoethanol (2-ME) also called as 2-ME-RSAT. This disassociates nonspecific IgM and improves test specificity; where diagnostic sensitivity may decrease. Recall Hunky and Dory (Table 2) from last year's April 2018 IVMA Update (4). An interpretation is suspect if a positive result on the RSAT screen and a negative result on the 2-ME-RSAT. Now, time and retesting become our allies. Based upon regulatory officials' assessment who may quarantine the facility and/or animal(s) put into isolation.

**Table 2.** Brucella canis test reaction(s) and "Interpretation" of the results. The 2-ME-RSAT is performed on Dory's serum sample after it has been treated with 2-mercaptoethanol (2-ME). "Result" is the outcome of the agglutination reaction on the slide: "Neg", no agglutination observed; "Pos", when agglutination observed. "Interpretation" is given only after 2-ME-RSAT results are recorded.

Animal ID	al ID Specimen		Test Name	Result	Interpretation	
Hunky	Serum	В	. Canis RSAT		Neg	
Dory	Serum	В	. Canis RSAT		Pos	

B. Canis 2-ME-RSAT

There are two possible outcomes following 2-ME-RSAT. One is "RSAT – positive and 2-ME RSAT - positive" which means the dog has circulating antibody, is likely infected and interpreted as 'positive' ("Pos"). The other possible outcome is "RSAT – positive and 2-ME-RSAT – negative" which means there may be cross-reactivity with another gram-negative bacterium or the dog is early in the course of infection. This combination of results is interpreted as 'suspect' ("Sus"). Resampling and retesting should occur 4-6 weeks later and include all animals with a "Suspect" initial test PLUS all animals with a "Negative". This builds a clearer medical understanding of the involved animals, especially to determine if the suspect dog is early in the course of infection.

# **ANNOUNCEMENTS:**

## **Upcoming University Holidays:**

Independence Day – Thursday, July 4th Labor Day – Monday, September 2nd

HATS will be receiving drop-offs as normal on Thursday, July 4th and Monday, September 2nd.

# **Bacteriology Pooling Policy**

For samples with an inherently high level of background flora (e.g., fecal samples of any species, swine nasal/oral fluid samples), the ISU VDL bacteriology section does not pool samples. Such types of samples are set up individually, reported individually, and charged individually.

## **UPS EZ-Ship Violations**

When using the ISU VDL EZ SHIP program, it is important to remove any and all labels from reused boxes prior to shipping. Any previous shipping labels cause UPS trouble in appropriately sorting and deeming packages as hazardous or not. These types of labels can cause violations which will incur extra charges issued by UPS.



