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Guidelines for taking diagnostic samples from pigs

Oral fluids

A series of best practices leaflets
developed in conjunction with
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Monitoring

Prognostic profiling with oral fluids may be used to monitor animals with signs of disease and to estimate the circulation of pathogens in swine population. Prognostic profiling is not a diagnostic procedure, therefore oral fluid should not be used to rule out infections.

Detection of antibodies (e.g., ELISA-based tests)—Oral fluid samples can be tested for the presence of antibodies against porcine reproductive and respiratory syndrome virus (PRRSV). Serological testing is not suitable for ubiquitous pathogens such as porcine circovirus type 2 (PCV2).

Detection of pathogen RNA/DNA (PCR-based tests)—The presence of pathogens that cause pneumonia, such as *M. hyopneumoniae*, PRRSV, swine influenza virus (SIV), etc., can be confirmed in oral fluid using PCR, but their presence does not necessarily correlate with disease.

Animal selection

Deciding which animals to take samples from depends on the desired outcome (keep in mind that for monitoring purposes other materials are likely to be more feasible).

- **Detection of infection**—Select animals with clinical signs of infection.
- **Absence of infection**—Select asymptomatic animals, then take samples from animals selected at random during a walk through the pens.
- **Tracking of infection status over time (i.e., longitudinal examination)***—Take the first samples on day 1 and repeat samples from the same animals 2 to 4 weeks later.
- **Determination of infection status in different groups (i.e., cross-sectional examination)***—Take samples from animals of different ages (e.g., 4, 8, 12, 16, 20, and 24 weeks of age).

Sample size

Due to lack of data on the diagnostic sensitivity of oral fluid sampling, final recommendations for an appropriate sample size have yet to be established. The sample size is dependent on the unit of observation, which cannot be more detailed than the pen level.

Assuming that all animals within particular pens have the same or at least a similar infection status, a head count,

* If serological testing is to be used, send all samples to the laboratory in one batch to avoid potential variation between different batches of test kits.

Sample size (continued)

followed by comparison of this number to the values in Table 1 may be used as a rough guide. The total number of animals tested by oral fluid should be equal to or higher than the appropriate number in the table. The minimum number of samples is 2, in order to lower the impact of a potential false-negative result in the laboratory.

Table 1

Number of samples needed for detection of disease (i.e., at least one infected animal has tested positive)			
	% diseased animals within a group		
	5%	10%	20%
Group size	Number of samples (95% confidence level)		
100	44	25	13
200	50	26	13
300	53	27	13
750	57	28	13
3,000	58	29	13

Sample sizes may vary based on in-herd prevalence level of a disease, the tested disease itself, confidence level of the outcome, the requested test method, and the purpose of the sampling.

Preparation

- Use a new, clean, and unbleached cotton rope of a diameter appropriate to the size and age of the pigs (approx. 1 cm for weaners and 2 cm for growers/finishers). Do not use synthetic ropes, since these will not absorb the same amount of saliva as cotton.
- The length of the rope depends on the means of fixing it in the pen—fixing under the ceiling requires a longer rope, for example, than knotting to the pen wall.
- One rope is sufficient to sample up to 10 to 15 pigs. If pens are stocked with more pigs, the number of ropes will need to be increased.
- Use a new rope for each pen. In the case of pulse feeding (e.g., liquid feeding systems), try to take samples before or at least 2 hours after feeding, because otherwise pigs are too inactive and will not pay sufficient attention to the rope.

Follow these 2 steps below:

1. Use a sterile collection tube for each oral fluid sample (5–12 mL). These tubes should not contain any

salts, etc. Go to steps 1, 2, 3, 4a, 5, and 6 in the sampling technique section for detailed procedures. We recommend delivering the fresh sample to the laboratory in cooled transport within 24 hours after sampling transport.

2. Or use 1 or 2 Applied Biosystems™ GenoTube Livestock nasal swabs—follow steps 1, 2, 3, 4b, and 6 in the sampling technique. For shipment of the GenoTube sample, it is not necessary to use cooled transport or time-critical delivery.

Sampling technique

1. Tie the rope in an area that is easily accessible for all pigs in the pen. This area should also be clean and not in close proximity to drinkers or troughs. The lower end of the rope should stop at the same height as the pigs' mouths when standing in an upright position. If more than one rope is used for a particular pen, affix them as far apart as possible to separate pigs into groups.

2. Leave the rope in position for 30 minutes. Extend the time to 1 hour when pigs are less active.
3. Carefully remove the rope (it should not touch the floor) and extract the oral fluids from the rope by inserting the lower end into a clean plastic bag or clean disposable plastic boot. Cut the end and then squeeze the rope in the bag so that the oral fluids accumulate in one corner.

4. Follow one of the 2 options below:
    a. Cut one of the bottom corners of the plastic and drain the oral fluids into a collection tube. A minimum of 2 mL is required for further analysis.

Sampling technique (continued)

b. Or if using a GenoTube swab—dip the GenoTube swab into the oral fluids in the plastic bag. Swirl it around in the oral fluids until the swab has been fully soaked with oral fluids. Put the swab back into the GenoTube container and close the lid. The recommendation is to have a minimum number of 2 samples in order to lower the impact of a potential false-negative result in the laboratory. Sample sizes may vary based on in-herd prevalence level of a disease, the tested disease itself, confidence level of the outcome, the requested test method, and the purpose of the sampling.

5. If there is significant dust, feed particles, or other material in the sample, the fluid should be centrifuged at 2,000 x *g* for 10 minutes (step not needed for the GenoTube swabs).
6. Label the tube or the GenoTube immediately with pen ID and compartment ID using a waterproof marker. Write numbers and letters clearly according to good clinical practice.

Storage

Store the sample in a refrigerator until shipment to the laboratory, which should be within 1 day. If this is not possible (e.g., in the case of a longitudinal assessment), freeze it at –20 to –80°C.

GenoTubes can be stored and shipped under room temperature conditions to the laboratories without time-critical deliveries.

Shipment

Before sending any samples, we suggest contacting the laboratory to confirm that there are validated tests for oral fluids available. Tests that have been established for serum or tissue cannot be used for oral fluids without further validation and adjustment, where required.

Material from diseased animals is usually classified as “Biological substance, category B” according to UN regulations (UN 3373). It must be shipped in compliance with national regulations and, at least for international shipment, in compliance with “Packing Instruction 650” specified by the International Air Transport Association (IATA). National regulations and IATA instructions may change over time. If you have doubt about the actual regulations, please ask your courier or the lab.

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Shipment (continued)

The sample should be accompanied by a case history and examination form, including:

- Name of veterinarian
- Name of farmer or herd owner
- Invoicing information
- Species and/or breed, and age of sampled animals
- Date samples were taken
- Number of samples
- Type of samples
- Identification/labeling of samples (correlation between numbers on the samples and ear tags on pigs)
- Specified test that should be performed, such as "quantitative real-time PCR for PRRSV" rather than just "PRRSV detection"; or "ELISA for detection of antibodies against PRRSV" rather than just "PRRSV detection"
- Results from any previous tests that do not need to be repeated

Good background information can help the laboratory conduct the most appropriate tests and provide advice in context.

For more information, contact your farm animal diagnostic testing laboratory, or go to **thermofisher.com/animalhealth**

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