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Guidelines for taking diagnostic samples from pigs

Tonsil scrapings and tracheobronchial swabs

A series of best practices leaflets developed in conjunction with Dr. Heiko Nathues, Royal Veterinary College, UK

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Diagnostic use

Detection of pathogens—The presence of *A. pleuropneumoniae* can be confirmed in tonsil scrapings and the presence of *M. hyopneumoniae* in tracheobronchial swabs. In either case PCR-based tests can be used to detect pathogen RNA/DNA. *A. pleuropneumoniae* can also be isolated using culture techniques.

Animal selection

Deciding which animals to take samples from depends on the desired outcome:

- **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 compartment. Keep in mind that for the purposes of monitoring (e.g., SPF status of herd in case of *M. hyopneumoniae*) other material and methods may be more feasible and reliable.
- **Tracking of disease 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 disease 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).

* 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

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

Number of samples needed for determination of disease prevalence (i.e., when an estimation of prevalence has to be made by the vet; confirmation will then be done by testing a particular sample size)

		Precision (95% confidence level)		
Group size	Estimated prevalence	±5%	±10%	±20%
200	10%	82	30	10
200	20%	111	47	15
200	50%	132	65	22
500	10%	109	35	10
500	20%	165	55	15
500	50%	217	81	24
3,000	10%	138	35	10
3,000	20%	246	61	15
3,000	50%	341	96	24

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

- Do not take samples from animals in extensively overcrowded pens—pigs may panic and hurt each other or the veterinarian during sampling.
- Make sure animals are properly restrained in an appropriate fashion by a competent person.
- Ensure there is enough light in the work area.

- Wear ear plugs or ear defenders.
- In case of pulse feeding (e.g., liquid feeding systems), do not take blood for at least 1 hour after feeding to avoid chylous serum.
- Use a sterile scraping spatula or covered swab of a diameter and length appropriate to the weight of the pig.
- Use a new spatula or swab for each pig.
- Use a sterile collection tube for each pig. Tubes should not contain transport medium or any salts if the sample is for PCR testing.

Sampling technique

1. Place a mouth gate or wedge between the teeth of the upper and the lower jaw to keep the mouth wide open; proceed with caution, since pigs will bite immediately if the device is not placed and adjusted properly.
2. Carefully insert the spatula and take a scraping from both pharyngeal tonsils, or alternatively insert the swab into the trachea and swab the surface of the trachea.
3. Slowly remove the device and place the tip in a designated tube.
4. Label the tube immediately with the animal ID (ear tag number) using a waterproof marker. Write numbers and letters clearly according to good clinical practice.

Storage

The sample should be stored in a refrigerator until shipment to the laboratory, which should be within 24–36 hours. If this is not possible and only PCR is required, freeze the sample at –20 to –80°C. Keep in mind that no further cultural examination is possible after freezing a sample.

Shipment

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.

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

- Name of veterinarian
- Name of farmer/herd owner
- Invoicing information
- Species/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)

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Shipment (continued)

- Specified test that should be performed, such as “nested PCR for *A. pleuropneumoniae*” rather than just “App”
- 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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