## applied biosystems



# Guidelines for taking diagnostic samples from pigs

Nasal swabs

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



#### Diagnostic use

Detection of bacterial respiratory pathogens—Nasal swabs can be tested by culture for the presence of *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida*, which jointly cause progressive rhinitis atrophicans (pRA). A very careful interpretation is recommended when commensals of the upper respiratory tract, such as *Haemophilus parasuis*, *Mycoplasma hyorhinis*, or *Streptococcus suis*, are found in nasal swabs; such findings cannot be used to determine the etiology of any respiratory diseases.

#### Detection of viral respiratory pathogens and Mycoplasma hyopneumoniae RNA/DNA (PCR-based

**tests)**—The presence of pathogens that are difficult to cultivate can be confirmed in nasal swabs by PCR. Detection of swine influenza virus (SIV) supports a presumptive diagnosis of influenza outbreak, whereas (no) detection of *M. hyopneumoniae* can be of interest in the context of monitoring SPF herds with suspicious clinical signs.

#### 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 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 at appropriate time intervals.
- 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

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)

	Estimated	Precision (95% confidence level)			
Group size	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.



Sucklers



Weaners



Growers/finishers

- Ensure there is enough light in the work area.
- Wear ear plugs or ear defenders.
- Use swabs of a diameter and length appropriate to the weight of the pig.
- The stem of the swab should be made of plastic, which is less likely to break than a wooden stem (pigs often make defensive movements during sampling).
- The tip of the swab should be flocked with a synthetic fiber (e.g., Dacron<sup>™</sup>) rather than with cotton, if PCR is the intended diagnostic test; cotton fibers do not release a sufficient amount of material swabbed from the nasal epithelium.
- For cultural testing, use swab containers with medium (e.g., Amies). For PCR testing, use swab containers without medium. If both methods are required, take two swabs from each pig and use the two different types of containers.

#### Sampling technique

- 1. Restrain the pig. Use an iron snare if needed. Pay attention to the correct positioning of the snare, which should always be beyond the first premolars.
- 2. Clean the nose with a dry piece of paper.
- 3. Push the swab deep into the ventral passage of the nose and leave it in this position for 3 seconds. To avoid bleeding, do not injure the nasal conchae by applying too much pressure.
- 4. Rotate the swab one third around the central axis and leave it again for 3 seconds.
- 5. Again rotate the swab one third around the central axis and leave it for 3 seconds.

NOTE: Steps 4 and 5 significantly increase the amount of material captured by the swab and thereby the diagnostic sensitivity of the whole method.

- 6. Place the swab in the appropriate container.
- Label the container 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  $-80^{\circ}$ 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

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#### Shipment (continued)

- 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 "nested PCR for *Mycoplasma hyopneumoniae*" rather than just "*M. hyo* 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.

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



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