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Guidelines for taking diagnostic samples from pigs

Bronchoalveolar lavage fluids

A series of best practices leaflets
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Diagnostic use

Detection of bacterial respiratory pathogens—Bronchoalveolar lavage fluid (BALF) can be tested by culture for the presence of a range of bacterial pathogens that cause pneumonia, including *A. pleuropneumonia*, *B. bronchiseptica*, *P. multocida*, etc. A careful interpretation is recommended when commensals of the upper respiratory tract, such as *H. parasuis*, *M. hyorhinis*, or *S. suis*, are found in BALF; such findings are often associated with contamination during sampling.

Detection of viral respiratory pathogen and *M. hyopneumoniae* RNA/DNA (PCR-based tests)—the presence of pneumonia-causing pathogens that are difficult to cultivate, such as *M. hyopneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV), and others can be confirmed in BALF by PCR.

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

For diagnosis of respiratory disease, a minimum of 5 pigs per (age) group that is affected should be sampled. If BALF is used for monitoring, the sample size usually has to be larger, e.g., 10 pigs or more per group. 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.

* 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.

Preparation

Depending on the body weight of the pigs, personal preferences, and capabilities, there are at least three different routes to access the lower airways of pigs. For the transoral route pigs should be anesthetized, and for the transtracheal route pigs must be anesthetized. In the case of pulse feeding (e.g., liquid feeding systems), do not anesthetize pigs for at least 1 hour after feeding to avoid cardiovascular shock.

	Transoral lavage (TOR)—Flasshoff (1996)	Transnasal lavage (TNR)—Nienhoff & Bossow (2004)	Transtracheal lavage (TTR)—Nienhoff et al. (2006)
Body weight	5 to 80 kg	60 to 120 kg	5 to 40 kg
Equipment	-Spatula (Miller F4) -Light -Endotracheal tube 4.5 x 220 mm / 6.5 x 290 mm -Lavage catheter 3 x 500 mm / 4 x 600 mm	-Iron snare -Lavage catheter 0.7 / 1.3 x >950 mm	-Permanent needle 12G, 2.7 x 50 mm -Nutrition tube 1.5 / 2.1 x 500 mm
Anesthesia	+	–	+
Invasiveness	–	–	+
Contamination *S. suis*	+	+++	–
Contamination *H. parasuis*	+	+	–

Ensure there is enough light in the work area, which should also be spacious. Use a sterile collection tube for each pig (5–12 mL).

Sampling technique TOR

1. Place the pig in the correct position, which is characterized by a straight vertebral column, front legs directed towards the hip, and rear legs directed to the elbows. The head should be lifted in a way that the epiglottis is on the same horizontal level as the hip joint.
2. Pull the tongue carefully straight out of the mouth using one hand.
3. With the other hand push the spatula towards the epiglottis and place the tip in the rima glottidis.

4. Insert a tracheotubus into the trachea and subsequently push the catheter through the tubus as deep as possible into the lung.
5. Instill a sterile sodium chloride solution (or PBS) and aspirate it after 2–3 breaths of the animal. The amount of solution should not exceed 0.5–0.7 mL per kg of pig.

Sampling technique TNR

1. Restrain the pig with an iron snare. 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 catheter through the ventral passage of the nose until the pharynx is reached.
4. As soon as the pig stops squealing, push the catheter forward through the wide-open rima glottidis into the trachea and then into the lower airways. Successfully reaching the trachea rather than the esophagus is easily assessed, since pigs show spontaneous coughing when the catheter touches the tracheal epithelium.
5. Instill sterile sodium chloride solution (or PBS) and aspirate it after 2–3 breaths of the animal. The amount of solution should not exceed 0.5–0.7 mL per kg of pig.

Sampling technique TTR

1. Move the pig into a lateral recumbent position and secure it by wrapping towels around the ventral and dorsal parts of its body.
2. Palpate the trachea and hold the larynx with one hand, while palpating the first tracheal rings beyond the larynx with the other hand.
3. Puncture the trachea with a permanent needle in a caudodorsal direction (approx. 1–2 cm caudal of the larynx) and help guide it toward reaching the trachea by aspirating air instead of blood.
4. Insert the catheter through the needle into the trachea and then push the catheter as deep as possible into the lung.
5. Inject a sterile sodium chloride solution (or PBS) and aspirate it after 2–3 breaths of the animal. The amount of solution should not exceed 0.5–0.7 mL per kg of pig.

Storage

The aspirated lavage fluid should be transferred immediately into a sterile sample collection tube with a twist lock. This tube should be labelled with the animal ID using a permanent marker. Write numbers and letters clearly according to good clinical practice.

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.

If cultural examination is required and transport cannot be organized immediately, the BALF should be centrifuged at 2,000 x *g* for 10 minutes. After discharging the supernatant, take a swab from the sediment and place this in appropriate transport medium (e.g., Amies) and store the swab at 8°C.

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

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Shipment (continued)

(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)
- Specified test that should be performed, such as "real-time PCR for *M. 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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