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Guidelines for taking diagnostic samples from pigs

Feces

A series of best practices leaflets
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Diagnostic use

Detection of pathogens by microscopy—Some protozoa and several helminth parasites or their eggs (e.g., *Ascaris suis, Balantidum coli, Strongyloides ransomi, Isospora suis, Oesophagostomum dentatum, Trichuris suis*, and others) as well as some viruses (e.g., rotaviruses, coronaviruses, and others) can be identified as suspicious using different microscopic techniques.

Detection of pathogens by cultural testing—Fecal samples can be tested by culture for the presence of a range of enteric pathogens, including *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Clostridium perfringens*, hemolytic *Escherichia coli (E. coli)*, and *Salmonella* species. Cultural testing is not suitable for *Lawsonia intracellularis* or viruses shed with feces. For *E. coli*, further characterization by PCR is recommended in order to identify AEEC (attaching and effacing *E. coli*), ETEC (enterotoxic *E. coli*) and STEC (Shiga toxin–producing *E. coli*). Similarly, PCR on bacterial cultures can be used to differentiate the subspecies of *Brachyspira* and test for the presence of the β2-toxin gene in *Clostridium perfringens* isolates.

Detection of pathogen RNA/DNA by PCR-based tests—The presence of pathogens that cause diarrhea, such as *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Lawsonia intracellularis*, porcine circovirus type 2 (PCV2), and *Salmonella* species, can be confirmed in feces. However, the detection of PCV2 alone does not confirm its relevance in clinical disease, and the detection of *Lawsonia intracellularis* should only be considered when a significant concentration has been shown by quantitative 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 2 to 4 weeks later.

* 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.

Animal selection (continued)

- **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

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 overcrowded pens—pigs may panic and hurt each other or the veterinarian during sampling.
- Ensure there is enough light in the work area.
- Pooling of samples on-farm is not recommended. If detection of *Salmonella* alone is of interest, pools of up to a maximum of five samples can be tested, but mixing of the samples should be performed in the laboratory.
- Take naive fecal samples for microscopy and PCR testing.
- Take rectal swabs for cultural testing.
- Use clean disposable gloves when feces are sampled from the rectum.
- Never collect feces from the floor—this would be a sample of the environment rather than pigs.
- Use new gloves for each pig.
- Use a sterile collection tube for each pig (5–12 mL). Tubes should not contain any salts, etc.
- Use a sterile swab for each pig. Swabs should be replaced in a tube containing transport medium—especially when isolation of *Brachyspira* spp. is the aim.

Sampling technique

1. Fix the pig by holding its tail with one hand.
2. Carefully insert one or two fingers of the other hand into the rectum and collect feces or insert the swab in the rectum, respectively.
3. Discard from gloved fingers into the collection tube until it is filled with 1–2 g of material.

A swab is used for sucklers

Weaner sampling

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

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.

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)
- Specified test that should be performed, such as "quantitative real-time PCR for *Lawsonia intracellularis*" rather than just "*Lawsonia* 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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