



Guidelines for taking diagnostic samples from pigs

Cervical swabs, semen, and urine

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

Diagnostic use

Detection of bacterial pathogens of the urogenital tract

—Cervical swabs, semen, and urine can be tested by culture for the presence of a range of bacterial pathogens that cause urinary tract diseases (UTDs) and/or reproductive disorders in either boars or sows. Pathogens include *Arcanobacterium pyogenes*, *Brucella* spp., *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Leptospira* spp., *Listeria monocytogenes*, *Pseudomonas* spp., *Staphylococcus aureus*, *Streptococcus equi*, and others. A careful interpretation is recommended when multiple commensals of the skin or the mucosa of the urogenital tract, such as *Staphylococcus aureus* or *Streptococcus suis*, are found in swabs or body fluids; such findings are often associated with contamination during sampling.

Detection of viral pathogens and *Chlamydia* spp.

RNA/DNA (PCR-based tests)—The presence of UTD or reproductive pathogens that are difficult to cultivate, such as ASF, *Chlamydia* spp., CSV, BVD, PHV-1, PCV-2, PRRSV, and SIV, can be confirmed in cervical swabs and semen 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 shed. Keep in mind that for the purposes of monitoring (e.g., SPF status of herd), very often other material is more feasible and reliable.

Sample size

In either case (UTD or reproductive disorder), at group level, a minimum of 5 pigs is recommended for sampling. If symptoms are not consistent in the affected group, select at least 5 pigs per “typical symptom”. For example, in a group with pigs showing with either cystitis or having experienced abortion, select 5 pigs showing cystitis as the main symptom and 5 sows that have recently aborted.

In the case where no symptoms of infection are present, you have to sample the number of pigs according to Table 1.

Table 1

Number of samples needed for detection of disease (i.e., at least one infected animal has tested positive)			
Group size	% diseased animals within a group		
	5%	10%	20%
	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

Cervical swab

- In the case of loose housing, selected sows have to be restrained for sampling. Make sure animals are properly restrained in an appropriate fashion by a competent person.
- Do not take samples in extensively overcrowded pens—pigs may panic and hurt each other or the veterinarian during sampling.
- Ensure there is enough light in the work area.
- Use a sterile speculum for each sow.
- Use swabs of a diameter and length appropriate to the age of the pig (gilt vs. sow). The stem of the swab should be made of plastic, which is less likely to break than a wooden stem (pigs often show defensive movements during sampling). The tip of the swab should be flocked with a synthetic fiber (e.g., Dacron™) 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.

Semen

- Only boars that have received appropriate training should be selected for sampling.

- Make sure that the collection pen is clean and free of extraneous items, which might distract the boar's attention from the phantom.
- Avoid noise, pain, fear, etc., which can negatively influence the boar's behavior for mounting the phantom.
- Ensure there is enough light in the work area.
- Use new sterile gloves for each boar.

Sampling technique—cervical swab

1. Clean the vulva with a dry piece of paper.
2. Drop some lubricant on the outer surface of the speculum and then slowly push it into the vagina.



Position the speculum against the cervix

3. Carefully insert the swab through the speculum until it reaches the cervix; leave it in this position for 3 seconds.



Insert the swab through the speculum

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. Label the swab container immediately with the animal ID (ear tag number) using a waterproof marker. Write numbers and letters clearly according to good clinical practice.

Sampling technique—semen

1. Use the “gloved hand technique” according to Almond.
2. Put pressure on the glans penis first and reduce pressure towards the beginning of ejaculation.
3. Carefully observe whether preputial fluid is ejected; because this sometimes contaminates the sample.

Sampling technique—urine

4. Enter the compartment, walk to the center, and force sows to stand up by clapping or shouting.
5. Wait a few minutes until approximately 1/3 of the sows that stood up spontaneously show micturition.
6. Quickly collect urine in the tubes from as many sows as possible.



Take a midstream sample if possible

Storage

Samples 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 any doubts 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

Shipment (continued)

- 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 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.

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