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Guidelines for taking diagnostic samples from pigs

Blood

A series of best practices leaflets
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ThermoFisher
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Diagnostic use

Detection of antibodies (e.g., ELISA-based)—Blood samples can be tested for the presence of antibodies that are associated with a range of pathogens, including porcine reproductive and respiratory syndrome virus (PRRSV), *M. hyopneumoniae*, swine influenza virus (SIV), *Salmonella* and *Trichinella* species, Prospect Hill virus-1 (PHV-1), pathogens in cerebrospinal fluid (CSF), and others. Serological testing is not suitable for ubiquitous pathogens such as porcine circovirus type 2 (PCV2), *H. parasuis*, *A. pleuropneumoniae*, *L. intracellularis*, and others. Bear in mind that antibodies, especially in young pigs, can be maternally derived and/or can occur after vaccination as well as after exposure to field strains of the pathogen.

Detection of pathogen RNA/DNA (PCR-based tests)—The presence of pathogens that cause viraemia, such as PRRSV, PCV2, CSF, etc., can be confirmed in blood.

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 (e.g., longitudinal examination)***—Take the first samples on day 1 and repeat samples from the same animals 2 to 4 weeks later (6 weeks later when *M. hyopneumoniae* is suspected in unvaccinated animals).
- **Determination of infection status in different groups (e.g., 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.

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.

Preparation (continued)

- 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 needle of a diameter and length appropriate to the weight of the pig.
- Use a new needle for each pig.
- Use a sterile blood collection tube for each pig (5–12 mL).
- Tubes may contain Na-heparin or Li-heparin but should not contain calcium salts if the sample is for PCR testing.

Sucklers

Weaners

Growers/finishers

Sampling technique

1. Puncture the vena cava cranialis (in piglets) or vena jugularis externa (in adults).
2. Slowly withdraw 4–8 mL of blood—avoid an excessive vacuum in the tube and high blood flow, as this can cause hemolysis.
3. Carefully remove the needle from the tube and use the designated cap to close the 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.

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)

Group size	Estimated prevalence	Precision (95% confidence level)		
		±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.

Sampling method

	Piglets	Medium	Large
System	Vacutainer™	Vacutainer	Syringe
Needle size	20 g	18 g	16 g
Needle length	1 in.	1.5 in.	1.5 or 2.0 in.
Vacutainer tube	10 mL	10 mL	10 mL
Vacutainer™ needle (two-sided, multi-draw) and tube holder	Yes	Yes	No
12 mL syringe	No	No	Yes
Comment	Draw the blood directly into the Vacutainer tube. The two-sided needle allows for the drawing of several tubes with a single needle stick. 10 mL max for newborn pig weighing ~ 3.0 lb. Generally, max volume for all pigs is no more than 1% of body weight.	Draw the blood directly into the Vacutainer tube. The two-sided needle allows for the drawing of several tubes with a single needle stick.	1. Draw blood into the syringe. 2. Remove needle from syringe. 3. Remove rubber stopper from Vacutainer tube. 4. Expel blood into uncapped Vacutainer tube. Note: Do not insert needle directly into rubber stopper of the Vacutainer tube—this will cause red blood cells to lyse and produce hemolysis. Hemolyzed serum samples may cause PCR inhibition.

Storage

1. Leave the sample upright at room temperature for at least 2 hours to allow the blood to clot completely.
2. After 2 hours, store the sample in a refrigerator until shipment to the laboratory, which should be within 2–3 days. If this is not possible (e.g., in the case of a longitudinal assessment), separate out the serum and freeze it at –20 to –80°C. Never freeze whole-blood samples because hemolysis will prevent any subsequent laboratory analysis.
3. If serum rather than whole blood is to be sent to the laboratory, then it should be separated by centrifuging the sample at 1,500–2,000 x *g* at room temperature for 10 minutes. If no centrifuge is available, then serum can be separated from the cruor after a further 10–12 hours by careful decanting. For a single PCR testing, a minimum of 1.5 mL serum should be sent to the laboratory; more serum should be sent if several PCRs and/or ELISAs are to be performed.

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, for international shipment, shipped 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 or herd owner
- Invoicing information
- Species and/or 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 to be performed, such as “quantitative real-time PCR for PRRSV” rather than just “PRRSV detection”; or “ELISA for detection of antibodies against 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.

For easier storage and shipment conditions, Applied Biosystems™ GenoTube Livestock Swabs with blood can also be used for PCR and ELISA tests. **GenoTubes allow quick drying of the sample, and shipment at room temperature to the laboratory for time-critical deliveries.**

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

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