

# Happy bite

## Instruction for sampling



1. Fix the rope in or above the pen at the pigs' shoulder height. Ensure it is well secured.



2. Allow the pigs to chew on the rope for 20 to 30 minutes to obtain a sufficient amount of oral fluid. Take down each rope and place in a single sealable bag. Wear gloves to prevent cross-contamination of the ropes.



3. Remove the securing cord from the rope and seal the bag. Press the oral fluid out of the rope by squeezing it tightly or stripping it. Collect **at least 10 ml** of oral fluid.



4. Get **new** gloves and tear the perforated corner of the bag. Transfer the oral fluid to a sterile tube and seal it. Take note: do not pool collected oral fluid samples.

5. Pack the tube in the small bag and seal it. Place all the small bags in the large pocket of the double bag. Add the submission form to the designated pocket. Send the sample **to the lab as soon as possible**. It is not necessary to freeze samples, but you must not store the oral fluid longer than one day.

### Tip:

Collecting oral fluid from multiple pens per age group provides more information than hanging a single rope per age group.



Unique test combinations  
for farm specific advice





GD Animal Health offers a new way to evaluate the health status of pigs: oral fluid testing. Oral fluid is easy to obtain by using special ropes in or above the pens. This means that samples are obtained from the pigs with less work and less stress.

#### Range of tests

- PRRSv PCR
- PCV2 PCR
- Influenza general PCR
- Influenza subtype specific PCR
- PRRSv ELISA
- PCV2 IgG ELISA
- Influenza A ELISA
- App ELISA
- Mycoplasma hyopneumoniae ELISA



#### Benefits of the new range of tests:

Testing for antibodies in oral fluid is now available for five pathogens: PRRS, PCV2, Influenza virus, App, *Mycoplasma hyopneumoniae*. Testing for PRRS virus, PCV2 virus and Influenza virus is also available. For Influenza, the general Influenza PCR, as well as a differentiating PCR is available for typing the three pathogens that occur in pigs (H1N1, H1N2 and H3N2).

#### Background of oral fluid testing

From 2008, the first scientific publications appeared on testing pig oral fluid for pig pathogens. The detection of KVP antibodies had been achieved earlier. American researches demonstrated that pig oral fluid is very suitable as sample material for detecting certain agents and antibodies. Since then, interest in these testing methods has increased further. The fluids tested (oral fluids) contain not only saliva, which is a reflection of serum, but also secretions from the mucosa in the cheeks and gums, serum and blood from wounds in the mouth, expectorated fluids from the airways, feed and water residues, and disposed oral mucosa cells.

#### Why test oral fluid?

Oral fluid testing is a very practical and accessible method in pig disease diagnostics. Lab results give a reliable picture of certain germs and antibodies that can occur in the majority of pigs in a pen. The use of oral fluid as sample material, instead of blood, for instance, has many benefits:

- Easy to obtain: the method requires very little work, and oral fluid can be obtained quickly and easily.
- Sample more animals at no extra cost: using just one rope the majority of pigs in the same pen can be sampled.
- Animal-friendly: avoids invasive and stressful methods, such as blood sampling.

#### Reliability of oral fluid testing

Various scientific publications have appeared on the use of oral fluid testing for monitoring. One key finding is that antibodies (IgG) against PRRSv can already be detected just 7 to 9 days after experimental infection with an American PRRSv strain [1]. In another study piglets were vaccinated with an American PRRSv strain, and placed individually four days after vaccination in 36 pens with PRRSv-negative piglets. The day after introduction, 64% of the pens indicated PCR-positive to PRRSv in oral fluid [2]. Furthermore, 77% agreement was detected between the results of oral fluid and blood tests [3]. In terms of PCV2, Prickett et al. [4] have confirmed that the virus is easy to detect in oral fluid and that IgG antibodies were first detected between 14 and 21 days after experimental infection.

#### How many ropes do you need?

##### Example for PRRS:

GD Animal Health has calculated the number of ropes necessary for oral fluid testing. The basis for this was the comparison of blood tests of all individual pigs in the pen with a oral fluid sample from the same pen. The calculation assumed a group of 100–500 weaned piglets of 8–10 weeks of age, divided over 1–5 compartments with 10 pens and 10 animals per pen (so in total 10 to 50 pens). Detection of a PRRSv infection requires:

- During the acute phase (fever, conjunctivitis, lung problems): testing of 2–3 ropes with PCR, one rope per pen, in selected pens with clinical symptoms,
- One to two weeks after the acute phase: testing of 2–3 ropes with PCR or ELISA, one rope per pen, in selected pens indicating or having indicated clinical symptoms.

#### Interpretation of results of oral fluid testing

Oral fluid testing is more suited to the confirmation of existing infections, especially with recent or existing clinical signs. A negative result does not necessarily mean that a herd is free of a certain pathogen/antibody. Where oral fluid tests are negative, we recommend either hanging more ropes in more separate pens or taking blood samples from individual animals. It is also important to test for other diseases that can cause the same clinical symptoms using oral fluid, blood samples or pathological examination. For questions on the interpretation of results, please don't hesitate to contact the veterinary service desk via +31 (0)570-63 33 91.



#### Literature

1. Kittawornrat, A., et al., *Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in oral fluid specimens using a commercial PRRSV serum antibody enzyme-linked immunosorbent assay*. J Vet Diagn Invest, 2012. **24**(2): p. 262-9.
2. Wang, C., et al. *Probability of detecting a singleton PRRSV viremic pig using pen-based oral fluid samples [samenvatting]* in Allen E. Leman Swine Conference. 2010.
3. Prickett, J.R., et al., *Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections*. J of Swine Health and Prod, 2008. **16**(2): p. 86 - 91.
4. Prickett, J.R., et al., *Prolonged Detection of PCV2 and Anti-PCV2 Antibody in Oral Fluids Following Experimental Inoculation*. Transbound Emerg Dis, 2011.



#### Which services does GD offer?

In addition to carrying out tests on oral fluid, GD Animal Health also supplies the Happy Bite rope packages and material for submitting cooled oral fluid (return package). Please visit the GD web store for these items: ([www.gd-winkel.nl](http://www.gd-winkel.nl)). The rope package can be ordered under number VDGE057. The code for ordering material for cooled transport is VDGE058. The submission form for oral fluid can be found in the Happy Bite rope packages, but can also be downloaded from our website (under Forms).

#### Submitting oral fluid samples

Proper handling of oral fluid samples is very important. Oral fluid is very sensitive and should be kept cool and sent to

GD as soon as possible. To carry out the full range of oral fluid tests, we require full tubes of at least 10 ml oral fluid.

#### How many pigs chew on a single rope?

In a group of up to 20 pigs 70 to 80% of the animals will have had contact with the rope within 20 minutes. For larger groups, this percentage will be lower, 40% for instance in a group of 50 pigs. So use one rope for 10 to no more than 20 pigs. If you are sampling young weaned piglets (5/6 weeks old), and the oral fluid yield is low, then you may unwrap the rope into its (three) constituent cords. You can then use one of these cords in each pen.

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