Principles of Oral Fluids Collection for the IDEXX PRRS Oral Fluids Antibody Test
Selecting the rope used in sample collection

1  Appropriate size and type of rope
   Studies have shown that raw cotton (unbleached and undyed) three-strand rope is best for diagnostic purposes. According to published studies, the rope should be at least ½-inch in diameter to ensure collection of an adequate sample volume.
   Using ropes made of nylon, hemp or other materials has been shown to significantly affect results of some diagnostic assays. In addition, the larger the rope, the higher the expected sample volume. A volume of 2.5 ml is sufficient for multiple diagnostic tests. For comparison, a 15-mL sample can be obtained from a ½- or 5/8-inch rope hung for about 20 minutes in a pen of 23-kg (50-lb) pigs or individual adult animals.

2  Where to purchase rope
   Rope made of 100% cotton can be purchased from stores that sell boating equipment or equine/bovine and pet supplies (tack or canine toys). IDEXX will offer an oral fluids collection kit in early 2014. Companies that make or supply rope are available online. Search for rope supply, wholesale cotton rope and the like. Hardware stores or other local vendors do not usually carry 100% cotton rope.

Placing the rope in pens and attracting pigs to chew

3  Placing the rope and collecting the samples
   Rope can be fastened directly to the pen or to a bracket attached to the pen, or it can be suspended over the pen (from an external device). The ends of the rope must hang at pig shoulder level and should be located away from waterers or feeders.
   After pigs are allowed to chew on the sampling rope for about 20 minutes, the rope is placed in a plastic bag. Oral fluids are extracted by pulling or squeezing on the rope while it is in the bag and allowing the fluid to collect at the bottom of the bag.
   If a collection tube is connected to the bag, cap and label the tube for laboratory submission. Otherwise, cut a corner of the bag and pour the oral fluid sample into a tube prepared for laboratory submission. For an example of the process, see the video noted in the references.
   If penning allows the knotting or hanging of rope, the rope can be tied so that both ends of the rope hang to allow interaction with pigs. If a bracket or an extension is available so the rope hangs freely in the pen, this is preferred because it enables more pigs to contact the rope at one time, but it is not required. Do not hang rope near waterers because this may dilute the sample.
   Important:
   • The oral fluid extracted from the rope is the sample for diagnostic testing! Do not submit rope to diagnostic laboratories as it will dry during shipment. The oral fluids must be extracted into tubes and sealed (with a lid) at the farm in order to be used as a diagnostic sample.
   • Do not pool oral fluids from different ropes.
   • The rope used to collect a diagnostic sample is not considered a vector for disease. Group-housed pigs already share feeders, waterers, floor space and so forth. The rope merely facilitates collection of the immunologic information from the animals.

4  Training pigs to chew on the rope
   When the rope is attached to the pen or to a bracket and is presented to the pigs, most growing pigs and adult animals will voluntarily come to the rope and begin to play with and chew it. However, recently weaned pigs (pigs 18–20 days of age) will need training. To train, unravel a three-strand rope. Tie one or two loose knots into each strand of the rope, and then toss this training
rope to the group of piglets in each pen that will be sampled. Although initially the piglets may scatter at the novel rope, they should quickly return and begin to nose and chew on it. As the pigs become more comfortable with the training rope, periodically drag it toward the pen gate or the location where the rope will be affixed. Once the training rope is close to the desired location, hang a new rope at that location at piglet shoulder height and unravel it, and then remove the training rope from the pen. As the hanging unraveled rope brushes the pigs, they will play with it and begin chewing.

Knots are tied in the training rope for two reasons: 1) knots prevent the rope from slipping through slatted flooring and 2) knots provide the pig with something more substantial to chew on. If pigs need to be directed to the suspended rope after the training rope is removed, use the training rope to entice them to the new piece of rope. Do not collect oral fluid from the training rope. It will take about 15–20 minutes to train recently weaned pigs. Once animals are trained, the sampling rope should be left attached to the pen for at least 20–30 minutes before oral fluids are extracted.

5 Using an attractant to encourage pigs to chew

Attractants are substances sprayed on or in the area of the rope to entice animals to investigate and chew on the rope. Sweet-smelling or sweet-tasting substances have been used on occasion to entice boars. Boar scent (synthetic boar pheromone) sprayed in front of sows stimulates excitement and activity for sow oral fluid collection. Apple juice or sugar water sprayed lightly on the rope has been successful, with no effects noted in assay performance. Take care not to soak the rope, however, as this does dilute the sample and may affect sensitivity. To entice sows, Hog Mate™ (a boar odor spray from ITSI Provisions) has been used with success when sprayed in the area where oral fluids collection will occur at a sow farm.

6 Length of time to leave the rope in the pen

Allow about 20 minutes for growing and adult pigs to sufficiently interact with the rope. Longer times may be necessary for wean-age pigs, but take care to extract the fluid immediately after the young pigs lose interest because the farrowing house and nursery environment are warm and ropes may begin to dry.

7 Percentage of pigs likely to chew on the rope and the implications for test results

Swine oral fluids research has been conducted in barns with pens of up to 30 pigs. In all studies, approximately 75% of the pigs in the pen had chewed on the rope after about 20 minutes. Because pen-based oral fluids are a composite sample, when using pen-based oral fluid samples, the results of the assay reflect the health status of the population, i.e., pen and barn. Even if one particular pig does not chew on the rope, because it is housed with other pigs a pathogen infecting that animal is shared with its pen mates.

Antibody response will be detected in the group as the disease spreads. In addition, the antibody status of an individual animal is less important when monitoring a population. However, oral fluid samples should not replace standard diagnostic workups, including necropsies, in cases of clinical investigations.

8 Tips for sampling pigs of different age groups

Oral fluid sample collection can be used with virtually all pigs of any age. Growing pigs and replacement animals are easiest to sample, as they are curious and interested in their environment. Little or no training is necessary for these animals.

Individually stalled or group-housed adults may be more challenging, but the success rate can be improved with strategic collection tricks such as sampling in the morning before feeding and using attractants. In addition, for individually housed animals, select more curious and active animals, and take advantage of the influence of curious neighbor animals—ropes can be hung in sequential stalls to maximize collection success.
Piglets ready for weaning (≥18 days old) are inquisitive about their environment and will readily chew novel things. Although it may be necessary to train these animals, hanging multiple single-stranded ropes near the front of the farrowing crate may entice both the sow and piglets to chew. If a sample is needed only from the litter, once training is successful, suspend new sampling rope strands farther from the sow.

Neonatal pigs are not recommended subjects for sampling, as they are only interested in nursing and thermoregulation.

**Collecting, handling and submitting samples**

**9 Determining which pens and how many pens to sample**

Selected pens should represent different areas of the barn, but otherwise rope should be hung randomly. Studies are underway to determine the best number of pens to balance diagnostic information and cost. Current studies suggest that in a single barn of 1,000–2,000 animals with 40–50 pens, 10 ropes provide enough relevant health information to observe and identify pathogen and antibody changes. Once sampling pens are designated, samples should be collected biweekly from the same pens, to minimize the interpretation of variation in results.

Swine oral fluids studies have been conducted using samples from barns containing up to 30 pigs per pen. Therefore, our recommendation is one rope per pen, for pens of 30 pigs or fewer. Where pens are larger (100 or more pigs), multiple ropes should be hung within the same pen in different areas to entice different groups of pigs. The samples from each rope should be submitted individually rather than pooled, as pooling may unintentionally influence diagnostic results.

Even though disease is easily shared within a pen of growing pigs, within a barn the spread of disease between pens varies depending on the facility’s ventilation system (e.g., natural versus tunnel), penning design (e.g., open gating vs. concrete dividers) and waste management method (e.g., gutters vs. slatted floors). For this reason, multiple pens within a barn should be sampled to obtain a more accurate picture of the immune status of the pigs.

Additional examples:

- In 1,000- to 2,000-head large-pen barns, hang multiple ropes in a pen (as described above) to ensure adequate population sampling.
- In smaller (e.g., 500-head) one-room barns, five ropes should be adequate.
- In barns with continuous flow production, choose the number of ropes to hang based on the number of pens representing different age groups of pigs.

When a farm site contains multiple growing-pig barns, all barns should be sampled in order to monitor disease. Although the pigs may be from the same source, disease dynamics within the population (as well as new disease introductions) may be different. In these situations, where the disease-monitoring focus is the site rather than the barn, useful information can be gained using fewer samples per barn (e.g., 3–5), as long as the samples are collected at least biweekly. In this way, information is gained about the PRRS dynamics of the entire site, although sensitivity at the barn level is reduced because fewer samples were submitted per barn.

**10 Recommended sampling frequency**

Weekly sampling is ideal, but biweekly sampling should be feasible in any production setting. If a production system is currently monitoring serology, samples are probably drawn on a monthly basis. However, to generate more useful, reliable and timely information from enzyme-linked immunosorbent assay (ELISA) results, samples must be taken more frequently—at least biweekly. Biweekly oral fluids sampling provides significantly more information regarding disease and herd immunity dynamics, which monthly serum sampling will not capture.
When to use oral fluid testing in place of serum testing

Whether oral fluids testing can replace serum testing depends on the intent of sampling. Oral fluids testing is extremely easy and useful for population monitoring. However, even though highly sensitive, the diagnostic sensitivity of the IDEXX PRRS Oral Fluids Ab Test for an individual animal at a single point in time is slightly less than that of the serum IDEXX PRRS X3 Ab Test. For individual animal testing (e.g., with genetic nucleus herds or boar studs, or to confirm PRRS antibody-negative status before shipping replacement gilts), use the IDEXX PRRS X3 Ab Test.

Oral fluids collection is easy, and therefore samples can and should be taken frequently (at least biweekly is our recommendation). The value of the oral fluids assay lies in frequent testing because:

1. There are fewer samples taken at each time point.
2. The samples are a composite (representing all pigs that chewed) and oral fluids by nature have a lower concentration of antibodies than serum.

Research has shown that in a pen situation, a single pen-based oral fluids sample has a significantly higher probability of detecting antibodies (and virus) than a random pig-serum sample from that pen, even when the prevalence in the pen is 4%.

Because of this, if production systems have been monitoring growing pigs for PRRS, switching to pen-based oral fluids sampling with the IDEXX PRRS Oral Fluids Ab Test will provide better information about the population, as long as monitoring is maintained faithfully and samples are run biweekly.
Lastly, oral fluids can be collected from individually housed animals or pens of animals. However, with individually housed animals the farm should collect the same number of individual oral fluids samples as they would have bled sows (or boars). Furthermore, the samples should not be pooled. The benefit of oral fluids sampling is improved well-being for the animal (compared to collecting blood) and reduced stress for the animal and caretaker.

15 Oral fluids test results compared to serum antibody test results

Oral fluids test results have higher sample-to-positive (S/P) values than serum test results. There is no direct correlation between the S/P values of serum and oral fluids.

S/P values considered “normal” for serum (i.e., 0.5–1.5) would generally compare to S/P values of 3.0–6.0 for oral fluids. Higher values (S/P ratio >6.0) are not uncommon, but they may indicate a recent or current PRRS virus infection. Biweekly oral fluids testing helps producers and veterinarians better understand and address PRRS circulation in the herd and provides more information for decision-making about PRRS control or elimination.

Some ELISA readers may give a reading of Overflow or ****. This indicates that the optical density (OD) and subsequent S/P calculation are outside the limits of the reader software. This reading error should not happen if xChekPlus® software is used, because xChekPlus software can provide S/P calculations even if OD values are outside the limits of the reader. Nevertheless, IDEXX Technical Services can assist in assessing and interpreting S/P values >4.0, which can appear with the IDEXX PRRS Oral Fluids Ab Test. High values are not surprising because of the sensitivity of the test; however, they are not directly interpretable.

For example, if an S/P ratio of 8.3 is reported for an oral fluids sample, the individual numeric value should be interpreted relative to:

- The group/barn mean—how does an S/P ratio of 8.3 for a pen fit within the variation around the mean of that group/barn?
- Consistency with the mean—if the mean is also high, the general interpretation of the group should be that an active PRRS infection may be circulating in the barn.
- Consistency with previous results—are the mean and high S/P ratios consistent when compared with previous sample results from the same group?

If these samples are the first of a group, a baseline set of data should be established with a series of 3–4 biweekly sampling episodes. As the oral fluids monitoring program continues with subsequent groups, there will be more information available as a basis for better planning and intervention and for better decisions about PRRS control or eradication.

As with serologic monitoring, do not assume that immune status is the same between pig flows from different sow farms. PRRS dynamics in growing pigs are directly related to virus load at the sow farm, maternal antibodies shared with the pigs and biosecurity practices—all highly variable factors. Finally, very high oral fluids S/P values, as is the case with high serum values, indicate high antibody presence. If high ratios continue over time, suspect PRRS instability in that farm or flow. Tracking antibody conversion over time in growing pigs provides better information for PRRS management.

16 Oral fluids polymerase chain reaction (PCR) tests versus antibody ELISA

A PCR test detects nucleic acid of the actual pathogen, whereas an antibody ELISA detects antibodies produced by the animal after it has been exposed to the pathogen. IDEXX is developing a PRRS PCR test.

Typically, PCR tests are requested during the acute phase of a disease to find the causative agent, while antibody testing is used either to confirm that a specific pathogen was the cause for clinical disease (by looking for a significant increase in antibody titers in convalescent sera or oral fluids) or to confirm that an animal has been exposed to a particular disease agent in the past.
Setting up an oral fluids monitoring program

17 Creating a new baseline for the IDEXX PRRS Oral Fluids Ab Test even when a baseline exists for the IDEXX PRRS X3 Ab Test

Even if a baseline is already established with the IDEXX PRRS X3 Ab Test, a new baseline for oral fluids must be created. However, the shape and trends of the oral fluids baseline should be the same as the shape and trends of the IDEXX PRRS X3 Ab Test. Because the antibody concentration in oral fluids is lower, the oral fluids assay must be more sensitive to detect the smaller amount of antibody; therefore, the magnitudes of the S/P ratios will be different.

18 Using xChekPlus® software to build a test-result graph

IDEXX Technical Services is working on a standard step-by-step process to make a mean S/P graph for oral fluids antibodies over time. xChekPlus software can do this kind of reporting now, but IDEXX is creating clear directions to allow veterinarians and production systems to make these valuable health-monitoring tools on their own.

19 Using oral fluids to monitor PRRS vaccination programs

The IDEXX PRRS Oral Fluids Ab Test can be used to monitor PRRS vaccination programs, as long as the PRRS vaccine is a modified live vaccine. The antibody response to a modified live PRRS vaccine is systemic, circulating in the blood and therefore present in the oral fluids of the animal. Both the IDEXX PRRS X3 Ab Test and the IDEXX PRRS Oral Fluids Ab Test detect antibody response to a modified live vaccine. Because oral fluids collection is easy and noninvasive, vaccine and vaccination-compliance monitoring are easily accomplished using oral fluids.

20 Setting up a monitoring program for a farm or production system

Monitoring programs are easy to set up. Initially, the sampling program should be designed to obtain baseline information. If the production system is a single sow-farm flow (or multiple sow farms flowing into a single growing-pig flow), monitoring programs can begin in both the growing-pig and sow locations. See the information above for sample size, location and frequency.

In some situations, a veterinarian may choose which pig flow or flows to begin monitoring, if he/she has a particular herd in which health has been difficult to manage. Oral fluids sampling is easily designed for a biweekly sample submission program.

Determining the number of samples is less defined (see “Determining which pens and how many pens to sample” above). Data should be collected as the mean (and variance/standard deviation) of the group (or barn), which can then be plotted and tracked over time. High S/P values tend to indicate recent infection and seroconversion (wild type or vaccine virus). Consistently high S/P values (i.e., S/P > 6) may indicate continuous virus challenge and suggest an unstable PRRS situation. When creating an oral fluids monitoring program for a sow farm, it’s important to understand and adapt the program based on housing design (pen or stall) and the number of barns and sows on the farm. Clearly, PRRS dynamics become less predictable as herd size and barn numbers increase. With oral fluids testing, sows in any stage of production (breeding, gestation or farrowing) can be sampled because the sample collection method causes no stress.
References