Diagnostics critical in effort to eradicate PRRS virus

By Nardy Robben, Product Specialist, Thermo Fisher Scientific

**Introduction**
Porcine reproductive and respiratory syndrome (PRRS) costs the global pig industry hundreds of millions of Euros each year. In Germany alone, losses in productivity due to PRRS are estimated at 116 million Euros annually. Proactive monitoring with diagnostic testing can significantly improve PRRS disease management.

Because of the complexity of a PRRS infection on a farm, the virulent nature of the virus, and rapid mutation rate, as well as challenges of interpreting diagnostic results when vaccination is used, intensive veterinary supervision is needed. Developing a strategic plan that includes proactive monitoring with diagnostic testing can significantly improve PRRS disease management.

Yet, recognizing PRRS virus in a pig herd can be difficult because symptoms often appear as general clinical signs. Pigs usually start coughing, are feverish, and don’t eat well. In sows, high abortion rates are often seen. It is prudent for the herd’s veterinarian to use diagnostic tests to rule out other diseases or co-infections before proceeding with a plan to address PRRS virus.

“Developing a strategic plan that includes proactive monitoring with diagnostic testing can significantly improve PRRS disease management.”

“Most veterinarians start by testing to see if a herd is positive or negative, and then they can build a plan for how to control PRRS in positive herds,” said Reinhard Bomkamp, production manager at TOPIGS-SNW GmbH. “In Northwest Germany, we have areas with high pig herd density, and many farms are positive for PRRS. Most use vaccination to reduce the economic impact of PRRS infections and to keep the virus in check.”
Especially in PRRS-free herds, it’s important to regularly check on whether the virus has been introduced or reintroduced onto the farm. Bomkamp recommends testing breeding animals monthly and sows every second month with both PCR and ELISA tests to confirm the absence of PRRS virus. The use of both molecular and serological tests gives the most complete picture of the herd status because each measures a different aspect of the PRRS virus.

“Walking the PRRS virus out of the herd requires intensive biosecurity measures and regular testing to monitor progress,” said Bomkamp. “The immediate economic advantage is clear: piglets do not need to be vaccinated, resulting in a cost savings of 1.50 Euro/piglet.”

A mutating PRRS virus
PRRS evades detection not only by causing nonspecific disease symptoms but also through the nature of the virus. PRRS has two genotypes: the European (EU) genotype and the North American (NA) genotype. The EU genotype PRRS strains change more rapidly than the NA strains, making them quite tricky to work with. At this time, they’re more diverse in the European regions and contain more subtypes than the NA strains.

“The biggest challenge with PCR is false-negative results caused by out-of-date primers (start sequences for the tests) that no longer match the highly variable virus. This is especially important for Eastern European strains,” noted Dr. Hendrik Nienhoff, swine veterinarian from SDG Lower Saxony (Germany).

“The use of both molecular and serological tests gives the most complete picture of the herd status because each measures a different aspect of the PRRS virus.”

Thermo Fisher Scientific continues to track and monitor global progress of both genotypes
Thermo Fisher is a global company with access to PRRS virus samples from different parts of the world, enabling us to follow and monitor genetic changes to the virus. Thermo Fisher PCR testing detects all currently known strains of the NA and EU genotypes, and our customers in Europe continue to request this test, in an effort to monitor and track any movement of highly pathogenic strains.

Diagnostic options
For accurate PRRS diagnosis, a combination of diagnostic methods is needed. The Thermo Fisher ELISA (Figure 1) is an excellent screening tool to detect antibodies against PRRS virus strains of both the EU and the NA genotypes. Because the antibodies can be detected as early as seven days after infection, ELISA is also used for routine herd monitoring.

A mutating PRRS virus
PRRS evades detection not only by causing nonspecific disease symptoms but also through the nature of the virus. PRRS has two genotypes: the European (EU) genotype and the North American (NA) genotype. The EU genotype PRRS strains change more rapidly than the NA strains, making them quite tricky to work with. At this time, they’re more diverse in the European regions and contain more subtypes than the NA strains.

“The biggest challenge with PCR is false-negative results caused by out-of-date primers (start sequences for the tests) that no longer match the highly variable virus. This is especially important for Eastern European strains,” noted Dr. Hendrik Nienhoff, swine veterinarian from SDG Lower Saxony (Germany).

“The use of both molecular and serological tests gives the most complete picture of the herd status because each measures a different aspect of the PRRS virus.”

Thermo Fisher Scientific continues to track and monitor global progress of both genotypes
Thermo Fisher is a global company with access to PRRS virus samples from different parts of the world, enabling us to follow and monitor genetic changes to the virus. Thermo Fisher PCR testing detects all currently known strains of the NA and EU genotypes, and our customers in Europe continue to request this test, in an effort to monitor and track any movement of highly pathogenic strains.

Monitoring should also include simultaneous real-time PCR testing. Through this approach, it’s possible to identify which pigs actually carry the PRRS virus and are shedding the virus on the farm. PCR detects the virus itself and provides clear proof of infection or non-infection, which is important to identify ahead of transporting pigs to another farm or barn. Even if animals have PRRS antibodies, it’s important to ensure they are not shedding when moving them because they can spread the virus and infect other animals, which can lead to clinical infections.

PCR can also differentiate between the EU and NA PRRS genotypes and in the case of a positive result, the nucleic acid can be used for sequencing, which offers highly detailed information about the virus. Sequencing can answer the key question: “Is it a positive test due to the modified live vaccination being administered, or do I have a new field infection?” Thermo Fisher has a workflow for targeted sequencing of either a full or partial genome.
**Sampling choices**

Diagnostics are commonly used to confirm clinical cases, but if producers and veterinarians want to control the disease and minimize economic losses, a proactive PRRS monitoring program will offer the best results. Routine monthly or quarterly testing using a combination of serology and molecular diagnostics as well as sequencing should be part of a producer’s health management plan. Utilizing diagnostics is the only way to be certain of which disease you’re dealing with, especially when respiratory and reproductive symptoms occur.

Dr. Nienhoff warns that it’s important to not only search for PRRS when problems occur on the farm, but for other conditions as well, because diseases like influenza can cause similar problems, may occur simultaneously, and could complicate the situation.

“Routine monthly or quarterly testing using a combination of serology and molecular diagnostics should be part of a producer’s health management plan.”

Proactive blood sampling without a suspected disease is often not allowed due to animal welfare regulations. Many producers therefore use the same blood sample for PRRS diagnosis that is being used for routine monitoring of notifiable diseases. More regular proactive sampling is possible with newer sampling matrices, such as oral fluids. Oral fluid sampling, which is animal-welfare friendly, is already standard practice in some countries. Pigs like to chew on the ropes used in oral fluid sampling (Figure 2), and it’s less stressful for them. Plus, it’s easy for the veterinarian or producer to administer.

Despite the less invasive nature of the procedure, veterinarians and producers in Germany have been slow to adopt oral fluid sampling for fear of missing infections. Several recent publications, however, have clearly validated oral fluid sampling results for a first screening at the herd level. (Table 1).

![Figure 2. Oral fluid sampling: pigs like to chew on the ropes, and it's less stressful for them.](image)

**Table 1. Oral fluid sampling recommendation**

<table>
<thead>
<tr>
<th>Prevalence of PRRSV (determined in serum)</th>
<th>Confidence level of 95%</th>
<th>Confidence level of 99%</th>
<th>Confidence level of 99.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>19</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>70</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Estimation of the number of pens to be sampled in order to detect at least one oral fluid positive, taking into account the prevalence present in serum. If the estimated prevalence of PRRSV in serum is higher than 50%, only one oral fluid sample per pen is needed to detect a positive sample with a confidence level of 95%.*
“With oral fluid sampling, we can achieve more information from significantly fewer samples. We use it to monitor negative herds,” Nienhoff said. “The advantages include easier sampling and better animal welfare. In groups of 15 animals, for instance, I can sample 60 animals with four ropes, one in each pen. In the case of serum testing, I would need to bleed 60 animals. There’s really no comparison as to which is easier on the animals and on me.”

“A veterinarian or producer can swab the oral fluid sample using the GenoTube swab, allowing the sample to be sent to the lab at room temperature without time-critical delivery.”

Due to the nature of the oral fluid samples, the challenge is that they must be shipped within 24 hours under cooled transport to the laboratory to prevent nucleic acid degradation. To overcome this challenge and improve handling of the sample, Thermo Fisher offers the GenoTube Livestock swab (Figure 3), designed to quickly dry and stabilize fluid samples. A veterinarian or producer can swab the oral fluid sample using the GenoTube swab, allowing the sample to be sent to the lab at room temperature without time-critical delivery.

**Optimized sample preparation**
Thermo Fisher offers an optimized sample extraction workflow to assist the customer in obtaining the best possible PCR result out of the sample. Regardless of the sample type, whether it’s an oral fluid sample collected with a GenoTube swab, a blood sample, or a tissue sample, the goal is to enable a high level of reliability in the results. The value of key outcomes is dependent on optimizing the full workflow for our customers—from the sample in the field all the way to the laboratory.