Use of processing fluids for PRRSV diagnostics
Carles Vilalta (cvilalta@umn.edu), Juan Sanhueza, Montse Torremorell, Bob Morrison

Keypoints:
- Using processing fluids as a diagnostic tool can help us to detect lower PRRS prevalence in the herd.
- Testicles and tails should be collected in a pail as they are potential spreaders of PRRS in the farrowing room
- We should target young parity sows for PRRSV sampling

Background
During the last few years, the improvement of sampling and diagnostics techniques has made sampling in farm an easier task. The use of blood pooling techniques and oral fluids are two examples of those improvements. However, veterinarians and producers are always looking for more sensitive, cheaper, and quicker techniques for sampling herds. One of the ways of achieving these goals would be to use routine chores, such as piglet processing, since castration and tail docking are part of the regular procedures in sow farms. We propose to use the processing fluids (PF), the liquid accumulated at the bottom of the pail when farmers collect tails and testicles during routine procedures, as a sample. The goal of this study was to evaluate the accuracy of the processing fluids by real-time PCR to assess Porcine Reproductive and Respiratory Syndrome virus (PRRSV) status in a sow herd.

Materials and methods
Sampling took place after an outbreak in a farm that was provisionally negative. 10 litters per week from different parties were selected and sampled at processing (3 days) during 8 consecutive weeks. Litters were conveniently selected in order to have a good spatial representation of the room and parities. All piglets of the selected litters were bled and tested in order to use that as the gold standard comparative sample. Testicles and tails from each litter were placed in a Ziplock bag. The tissues remained in the bag for at least two hours. Afterwards the fluid was removed with a sterile pipette and placed in a sterile serum tube. Both, blood and PF, were centrifuged at the farm and transported to the lab under refrigeration. All samples were tested by real-time RT-PCR.

Results and discussion
A table 2x2 (Figure 1) was built comparing serum status and PF status. A litter was considered positive if at least 1 of its piglets was positive in serum. Positives were categorized based upon a negative Ct value threshold lowered to 37.5 instead of the conventional 40 Ct, in order to include in the analysis samples that were in the suspect range, 35 to 40 Ct. Sensitivity (95% Confidence Interval (CI)) and specificity (95% CI) of the technique were 83% (63-95%) and 92% (82-98%) respectively. The agreement between both types of sampling was good (Kappa: 0.76 (95% CI: 0.53-0.98%)). 4 false positives and 4 false negatives were detected. The serum of the piglets in the false negative had a low virus load (high Cts) and when those were diluted the Ct of those samples fell in the negative range. Even though the transfer of the PF were made with extreme care some cross contamination might have been possible as we observed 4 false positive results. There was a statistical difference (p<0.05) between the of positive PF when comparing low parity groups (first and second farrow) vs high parity groups (more than second farrow). The lower parities were those with higher percentage of positives animals. This is in agreement with what Cano et al. (2008) found when trying to identify risk indicators associated with a higher probability of detection of PCR-positive piglets. PF showed to be positive even though only one piglet in the litter was positive showing that the PF are a very sensitive tool to capture what is happening in the litters, and therefore in the herd. At the same time these results highlight the importance of collecting testicles and tails in order to reduce the dissemination of the disease. Additionally, it was possible to recover and sequence PRRSV from the PF specimen.

One limitation of this study is that only samples from one farm are represented.

Conclusion
Processing fluids are an effective sample to detect PRRSV in piglets, even after a significant time since the outbreak (~ 6 months), especially in litters from young parity sows.

Acknowledgements
We would like to thank Deb Murray for sharing the idea with us, New Fashion Pork for providing the farm and Boeringher Ingelheim for the funding.

Cano et al. (2008) 39th Annual Meeting of the AASV.

<table>
<thead>
<tr>
<th>Litter status (serum)</th>
<th>+</th>
<th>-</th>
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<tbody>
<tr>
<td><strong>Processing fluids status</strong></td>
<td><strong>Litter count</strong></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>49</td>
</tr>
</tbody>
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Figure 1. Table 2x2 of the litter status using the serum as a golden standard.