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Lauritsen, Klara Tølbøl; Sørensen, Nanna Skall; Klausen, Joan; Lind, Peter

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Novel ELISAs for differentiated detection of antibodies against either PRRSV EU or US in oral fluid.

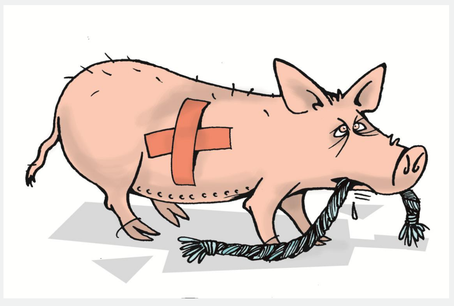
Klara Tølbøll Lauritsen[1], Nanna Skall Sørensen[1], Joan Klausen[1], Peter Lind[1]

[1] National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark, ktla@vet.dtu.dk

Conclusion: **Based on these data the intention is to continue the validation of this test system for differentiated detection of PRRS antibodies in oral fluid. OF-diagnostics will be a useful supplementary tool to the otherwise serum based surveillance of PRRSV EU and US in Danish swine herds.**

Background: In the Danish SPF system PRRSV surveillance is based on the ability to differentiate between the American (US, Type 2) and the European (EU, Type 1) strain of PRRSV. The blocking ELISAs used in this SPF-surveillance are only validated for serum (Sørensen et al., 1998). Based on the same antigens, indirect ELISAs for PRRSV EU and US were developed for analysis of oral fluid (OF) samples.

Materials: Samples for the validation were obtained from PRRSV positive and negative Danish herds. 281 OF pen pools were collected by hanging a rope in selected pens. From same pens blood was drawn from all pigs in each OF sampled pen, resulting in 2551 sera in total. The selected pens represented pigs ranging from 15 to 100 kg.



Methods: The sera were tested in the PRRS blocking ELISA used in the surveillance (Sørensen et al., 1998), and these results were the gold standard for the novel OF ELISAs: A PRRSV-positive pen was defined as a pen with at least 50% pigs positive in the blocking ELISA.

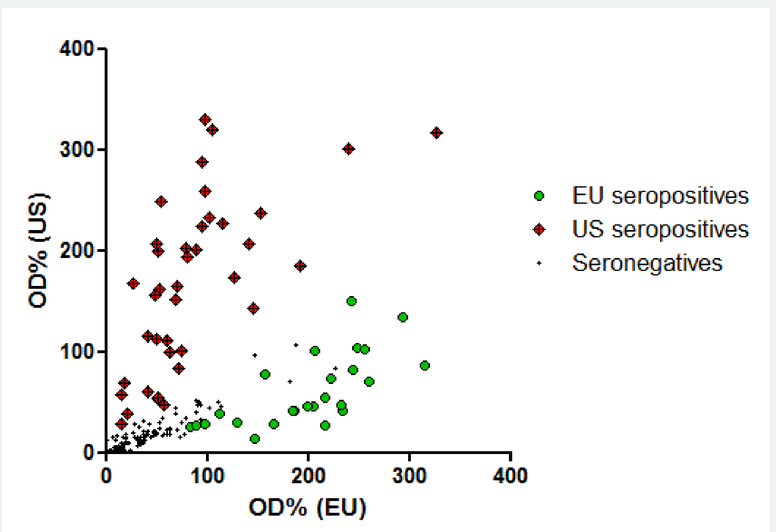


Figure 1: Plot showing oral fluid samples tested in the new OF ELISAs. US results (OF diluted 1:5) plotted against EU results (OF diluted 1:10).

Colour codes of symbols are referring to the serum gold standard: EU seropositive OF (**green**) = 100% of serum samples from the pen were EU-positive in the blocking ELISA. US seropositive OF (**red**) = 100% of serum samples from pen were US-positive in the blocking ELISA. Seronegative OF samples (**black**) = 100% of serum samples from the pen were negative in blocking ELISA.

Results: As shown i Fig 1, there was an obvious clustering into three populations, when testing expectedly positive and negative OF samples in the two OF ELISAs. This indicates that the tests can differentiate between EU and US positive samples.

In the novel US OF ELISA, choosing a pen specificity of 0.97, leading to a cut off value of 84 (calibrated OD value), the herd sensitivity with 10 pens sampled and a within herd pen prevalence of 0.2 would be 0.83. Likewise in the EU OF ELISA, with a pen specificity of 0.97 and a cut off value of 219 (calibrated OD value), herd sensitivity would be 0.78 with the chosen pen specificity and sampling 10 pens in a herd, the herd specificity will be 0.74 for both ELISAs.

As expected, a slight cross reactivity was found between the EU ELISA and the US ELISA (Fig 2). However, use of the abovementioned cut offs results in a reasonable specificity towards the heterologous strain in the two ELISAs. Thus specificity to the US strain in the EU-positive herds, is 74% and specificity to EU in the US herds, is 90%.

Discussion: The herd specificities may appear low – but when calculating these an assumption is made that all samples taken in one herd are independent on each other. This is naturally not the case in reality, therefore the herd specificity is expected to turn out to be higher in practice. We are going to test more paired OF/serum samples, to get to know the test even better. Further work is also to be done concerning description of guidelines for choosing sample size and performing safe diagnostics. Contrary to serum, OF is a highly variable material due to natural variation, risk of contamination and dilution (*Fig 3*). Collection should thus be as standardized as possible Switching from serum to OF is a way to intensify the sampling routine within a surveillance, without an excessive rise in analysis costs.

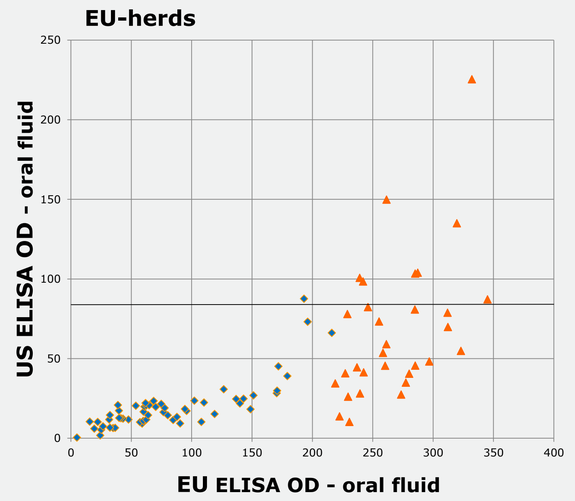


Figure 2: Figures showing all OF samples where at least one of the pigs in the pen had a positive serum sample (EU or US depending on graph). No samples from sero-negative pens have been included in the two graphs. Positive OF samples are shown as orange triangles. The cut offs of the heterologous ELISAs are indicated by the black line in each graph. These two graphs show that there is only little cross reactivity between the two tests: Only few of the OF samples that were positive in one ELISA were also positive in the other with the chosen cut offs. (Note that the sensitivity and specificity have been calculated based the definition of a positive pen as containing 50% seropositive or more in the pen).

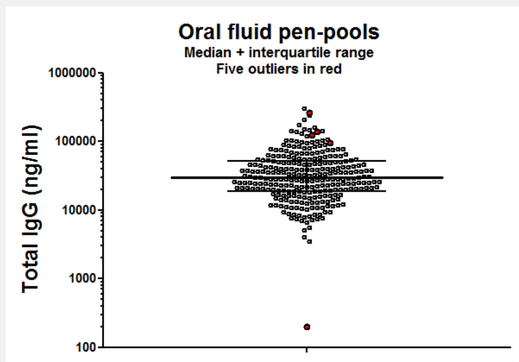


Figure 3: Scatter plot of total IgG levels measured in the OF samples collected within this project. We see a large variation in IgG-level between OF samples (i.e. pen pools). Outliers, giving very high or low responses in the PRRS ELISAs, are highlighted in **red**. IgG was measured in a total IgG sandwich ELISA.

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References:
1) Sørensen KJ, Strandbygaard B, Bøtner A, Madsen ES, Nielsen J, Have P. 1998. ”Blocking ELISA's for the distinction between antibodies against European and American strains of porcine reproductive and respiratory syndrome virus.” Vet Microbiol. 60, pp169-77.