Placental Umbilical Cord Sampling for Porcine Circovirus Type 2 and the effects of pooling on sensitivity of polymerase chain reaction results

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INTRODUCTION

Detection of Porcine Circovirus Type 2 (PCV2) in individual Placental Umbilical Cord Serum (PUCS) samples has been shown to be comparable to other sample types (e.g., colostrum, pre-suckle serum, fetal tissue) to monitor PCV2 sow herd stability. However, testing of individual samples is costly, and a frequently used method to reduce testing costs is sample pooling. The purpose of this study was to estimate the impact of PUCS sample pooling on PCV2 polymerase chain reaction (PCR) detection sensitivity.

MATERIALS AND METHODS

- Ninety-three PCR results per pool size were needed to detect a 20 % difference in sensitivity (i.e., 50 % vs 70 %) as significant at an alpha probability ≤ 0.05 and power ≥ 0.8.
- All samples were collected from Midwest United States breeding herds.
- Expelled placental tissues were gathered, placed in a refrigerator, at least three umbilical cords per placenta were collected and expressed into a serum seperator tube to make a single placental sample.
- A PCV2 rtPCR (HMC, Ames, IA) was run on each aggregate placental sample.
- Positive placental samples with Cq values ranging from 29.77 – 39.96 were categorized
  - High virus concentration (Cq < 34.41)
  - Middle virus concentration (Cq between 34.42 and 36.22)
  - Low virus concentration (Cq > 36.25)
- Using known placental sample results, pools were tested on PCV2 rtPCR
  - One positive sample plus one negative sample (2:1)
  - One positive sample plus two negative samples (3:1)
  - One positive sample plus four negative samples (5:1)
- The experimental unit was the placental sample rtPCR result, and pools that contained a PCR positive placental sample was considered as the positive gold standard for calculating relative sensitivity.

CONCLUSION

Pooling of PUCS-based placental samples reduced diagnostic detection sensitivity of PCV2 by rtPCR. As pooling increased, detection rates decreased. Further, the higher the initial Cq, the lower the detection rate for the middle and high Cq groups, i.e., pooling one middle or high Cq positive placental sample with one or more negative samples decreased detection rate in all pool sizes. These results can be used as a basis for further development of an optimal sampling protocol.

Figure 1: Total % Positive PCV2 PCR

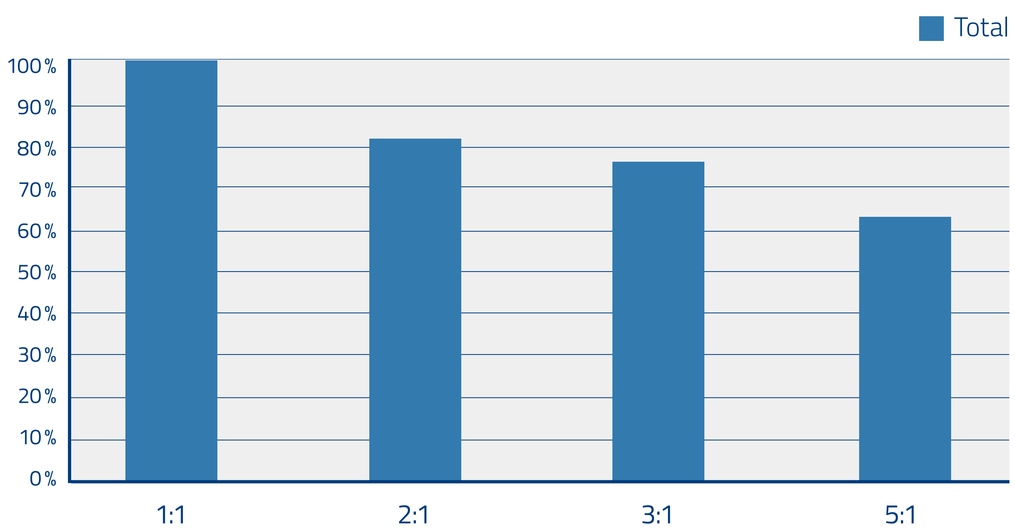

Figure 2: Effect of pooling PUCS on PCR sensitivity

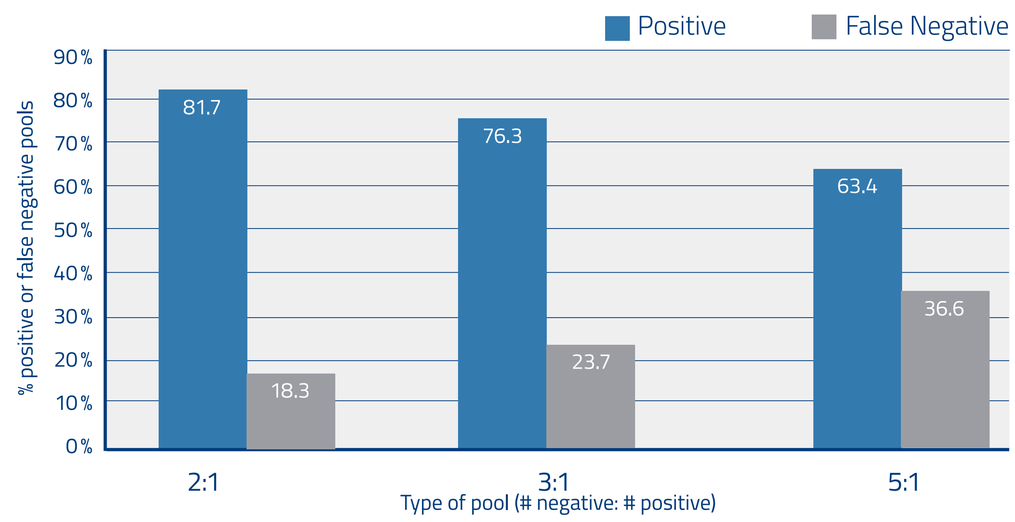

Figure 3: Placental Samples Positive by Cq Range

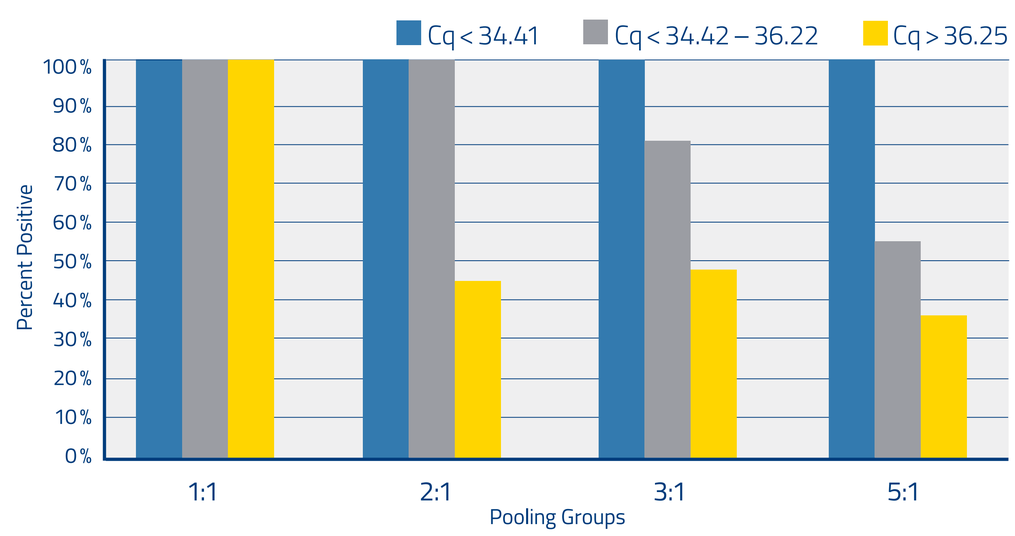

Table 1: Effect of pooling and median CT