

OPTIMIZING POOLING STRATEGIES FOR PRRSV SURVEILLANCE: RT-PCR DETECTION LIMITS FOR PRRSV1 AND PRRSV2

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INTRODUCTION

A Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) monitoring program is key to its control^{1,3,4}. Serum from due-to-wean pigs tested in pools is the recommended method by the American Association of Swine Veterinarians (AASV) for breeding herd monitoring and PRRSV status classification¹. Therefore, this study aimed to evaluate the detection limit of RT-PCR by pooling PRRSV (PRRSV1 and PRRSV2) positive serums with different levels of viremia and evaluate the dilution effect of pooling on initial Cycle threshold (Ct) values.

MATERIALS AND METHODS

A total of 47 of PRRSV-1 and 33 PRRSV-2 positive samples were analyzed by Reverse-transcription PCR (RT-PCR) and categorized into the following Cycle threshold (Ct) range groups: 18-25, 25-30, 30-35, and 35-38. Samples were serially diluted simulating 5, 10, 30, 60 and 120 pools, as it is represented in Figure 1.

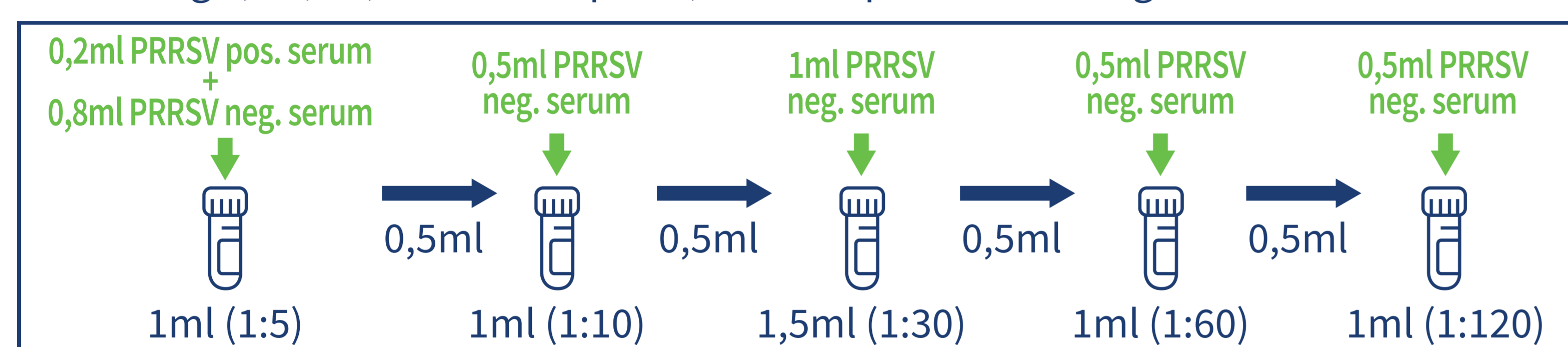


Figure 1. Dilution protocol simulating the pooling of one positive serum within pools of 5, 10, 30, 60 and 120 serums

Viral RNA extraction and quantification was performed following the manufacturer's instructions kit (MagMAX™ CORE Nucleic Acid Purification Kit and VetMAX™ PRRSV EU & NA 3.0 Kit). Only samples ≤ 38 were considered positive for this study.

Sensitivity was estimated for each Ct group at each dilution range. Furthermore, a linear mixed model was calculated to estimate the increase in Ct values by each dilution. All dilutions were transformed into base 10 logarithms scale. Data analysis was performed using R software (version 4.4.1, R Core Team).

RESULTS

Samples with Ct < 30 maintained a 100% sensitivity in all the dilution groups, excepting one sample that tested false negative in the 25-30 Ct group at 1:120 dilution for PRRSV2. On the other hand, samples with initial Ct > 30 had a decrease in sensitivity whilst increasing the pool size in both PRRSV1 and PRRSV2. Difference between species were probably explained for the number of samples used in that group.

Group	Pool Size				
	5	10	30	60	120
18-25	100.0% (14/14) (77-100%)	100.0% (14/14) (77-100%)	100.0% (14/14) (77-100%)	100.0% (14/14) (77-100%)	100.0% (14/14) (77-100%)
25-30	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)
30-35	100.0% (13/13) (75/100%)	84.6% (11/13) (55/98%)	76.9% (10/13) (46/95%)	46.2% (6/13) (19/75%)	38.5% (5/13) (14/68%)
35-38	66.7% (6/9) (30-92%)	22.2% (2/9) (3-60%)	11.1% (1/9) (0-48%)	11.1% (1/9) (0-48%)	11.1% (1/9) (0-48%)
Total	93.6% (44/47) (82-99%)	80.9% (38/47) (67-91%)	76.6% (36/47) (62-88%)	68.1% (32/47) (53-81%)	66.0% (31/47) (51-79%)

Table 1. PRRSV-1 Sensitivity (%) by RT-PCR CT group and dilution, number of positive samples out of the total per group, and 95% confidence interval.

Group	Pool Size				
	5	10	30	60	120
20-25	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)
25-30	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	100.0% (11/11) (72-100%)	90.9% (10/11) (59-100%)
30-35	45.5% (5/11) (17/77%)	36.4% (4/11) (11/69%)	9.1% (1/11) (0-41%)	18.2% (2/11) (2/52%)	9.1% (1/11) (0/41%)
Total	81.8% (27/33) (64-93%)	78.8% (26/33) (61-91%)	69.7% (23/33) (51-84%)	72.7% (24/33) (54-87%)	66.7% (22/33) (48-82%)

Table 2. PRRSV-2 Sensitivity (%) by RT-PCR CT group and dilution, number of positive samples out of the total per group, and 95% confidence interval.

The linear mixed model equation for each species was calculated, considering sample as a random effect:

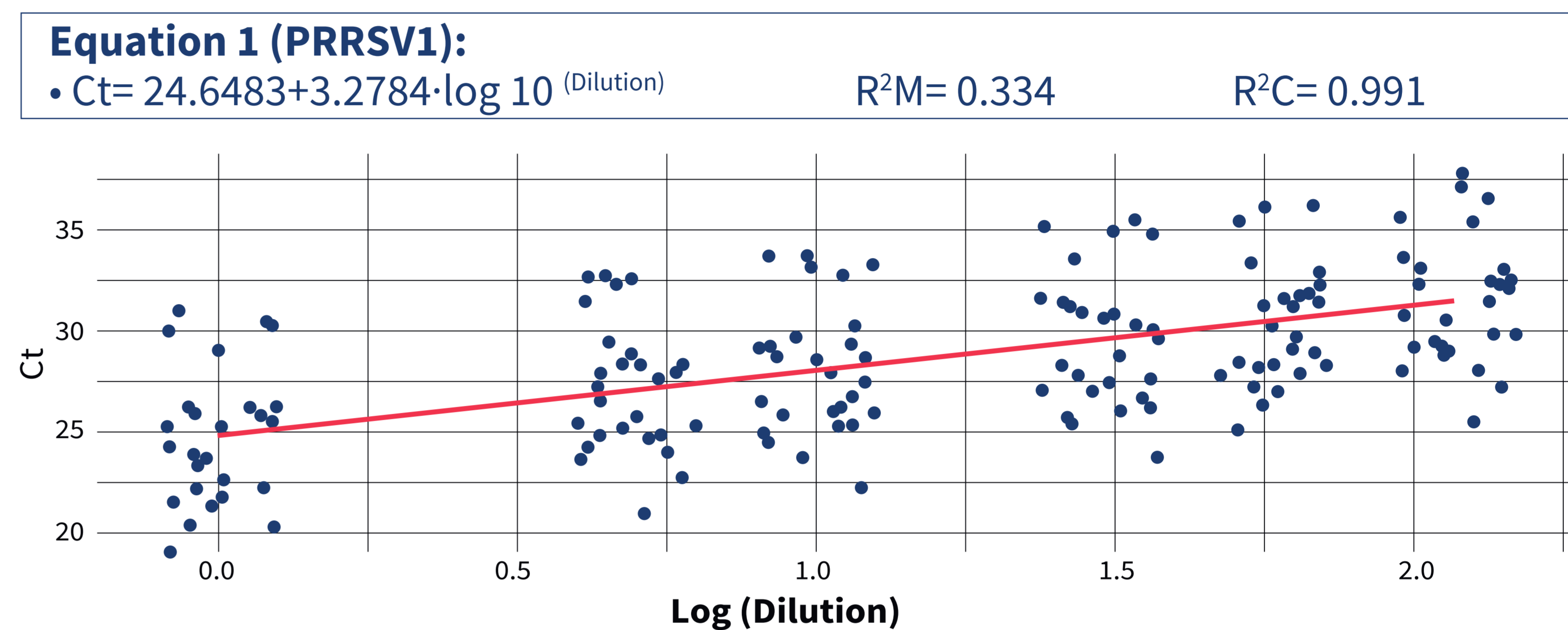


Figure 1. RT-PCR Ct values of initial and pooled serum samples, different dilutions expressed as Log10. Fitted linear mixed model for PRRSV-1 Results.

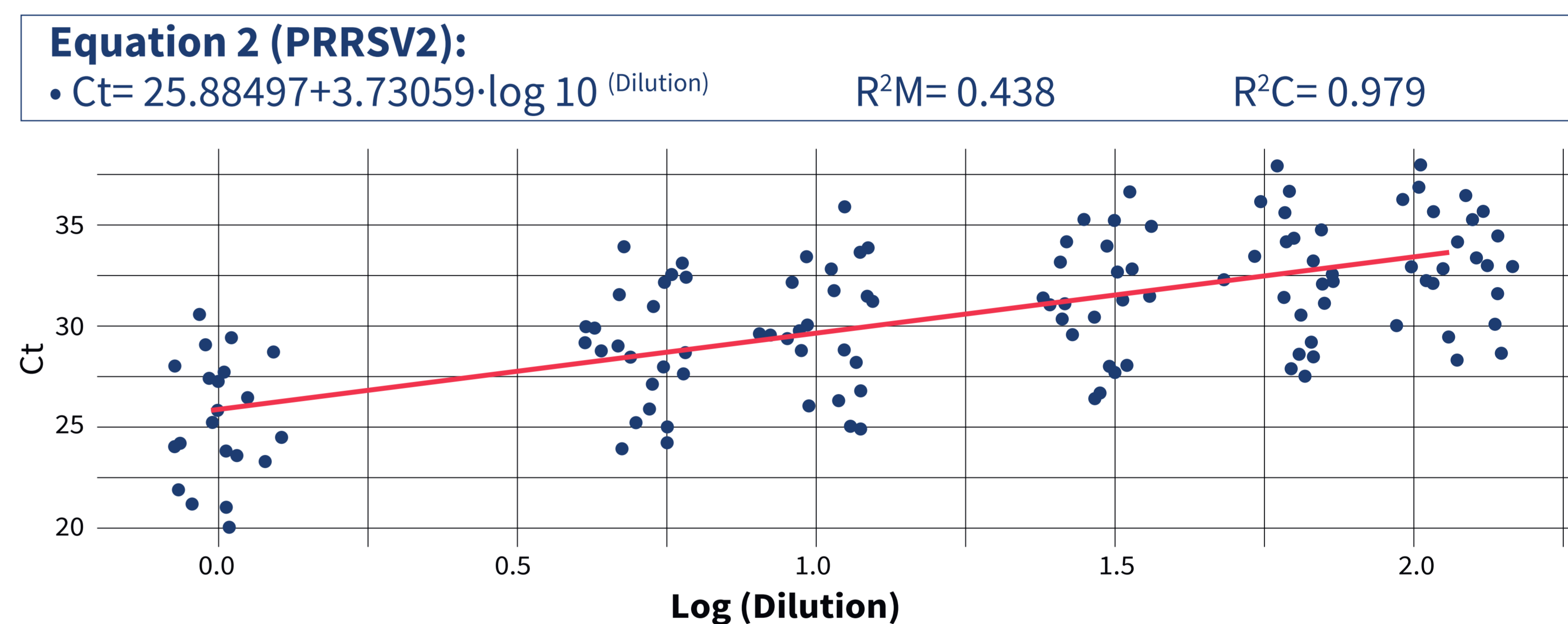


Figure 2. RT-PCR Ct values of initial and pooled serum samples, different dilutions expressed as Log10. Fitted linear mixed model for PRRSV-2 Results.

DISCUSSION AND CONCLUSIONS

Understanding the effect of pooling on RT-PCR PRRSV positive samples is key to implement an effective surveillance strategy, especially in low prevalence scenarios¹. Results from this study confirm that; pooling at least 5 serums have either no effect on sensitivity on serums with Ct values below 30, or a moderate effect on Ct values over 30 for both PRRSV species. This sensitivity results are consistent with previous studies^{2,3,4}. This indicates that the sensitivity of pooled samples depends on the viral load of the positive sample. Considering new highly pathogen strain scenarios in some countries/regions, where infection with PRRSV results in a high viral load in serum, pooling can be a valid strategy to reduce cost and still detect the early onset of the disease. Based on our model, the maximum number of dilution possible before overcoming the 38 Ct threshold varied depending on the initial Ct value. Thus, considering the worst-case scenario defined as a single positive sample with a Ct value of 35 the maximum allowable dilution for PRRSV-1 and PRRSV-2 would be 1:8 and 1:6, respectively. Pooling serum samples compensates the loss of individual sensitivity with the benefit of a larger sample size allowing to monitor more animals at reduced costs.

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