



PEDV Differentiation Real-Time PCR Fact Sheet

Type of assay: Real-time multiplex polymerase chain reaction (qPCR)

Manufacturer: N/A (developed in-house)

Purpose of the assay: To certify absence or presence of Porcine Epidemic Diarrhea Virus (PEDV) virulent and variant strain RNA in porcine samples. The variant strain of PEDV is the clinically less severe form of the disease.

Species: Porcine

Validated Specimens: Intestines, oral fluid, feces, feces, fecal swabs, and environmental swabs (biological, and non-biological). Other specimen types can be submitted but results will be reported with a disclaimer stating those sample types have not been validated in our lab).

Cost: \$31 per sample

Turn Around Time: 5 business days

Testing Schedule: Once per week, on Fridays

Analytical Sensitivity (1:10 dilution series, 2 dilutions beyond endpoint, tested in triplicate):

- Reportable range: Positive Ct \leq 36; Suspect Ct 36.01 – 39.99; Negative Ct $>$ 40
- Virulent PEDV: 25 viral copies/ μ L (obtained from a synthetic sequence)
- Variant PEDV: 25 viral copies/ μ L (obtained from a synthetic sequence)

Assay precision (presented as the coefficient of variation, or CV %):

- Within-assay
 - o Virulent: 2.2%
 - o Variant: 0.9%
- Between-assay
 - o Virulent: 4.7%
 - o Variant: 3.6%

Precision is the variability in data from replicate determinations of the same homogenous sample under normal assay conditions. Within-assay precision includes a single sample tested five times in a single assay. Between-assay precision involves aliquots of the same sample tested in at least 10 independent assays over multiple days. Perfect precision would result in a CV of 0%; our acceptance criteria are within-assay precision $<$ 10% and between-assay precision $<$ 20%.



Analytical Specificity:

- 100% (assay was negative when tested for 42 other viruses and bacteria)

Diagnostic Sensitivity and Specificity:

- | | |
|--------------------------|--------------------------|
| - Diagnostic Sensitivity | - Diagnostic Specificity |
| Virulent: 100% | Virulent: 100% |
| Variant: 100% | Variant: 100% |

Diagnostic sensitivity (DSe) and diagnostic specificity (DSp) were determined by comparing 161 samples (across various sample types) to other PCR assays. Sanger sequencing provided confirmation in selected samples representing samples positive for each strain individually, or dual infected.

Comparison data for negative samples were generated using clinical results obtained in the UMN VDL Molecular Diagnostics section using the Qiagen PEDV / PDCoV / TGEV triplex PCR. Positive samples were first identified in the same manner as the negative samples; the resulting differentiation results were confirmed using a conventional, gel-based PCR for these two PEDV strains. The gel-based PCR was verified by Sanger sequencing in representative samples (including Virulent positive, Variant positive, and dual positive).

***NOTE this assay does not include Porcine Deltacoronavirus (PDCoV), or Transmissible Gastroenteritis Virus (TGEV) like our routine PEDV / PDCoV / TGEV triplex assay. We recommend first testing samples with the PEDV / PDCoV / TGEV triplex PCR to confirm PEDV status before requesting the PEDV differentiation PCR.**