



Porcine Circovirus Types 2 and 3 (PCV2/3) Real-Time PCR Duplex Fact Sheet

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

Manufacturer: QIAGEN

Purpose of the assay: To certify absence or presence of PCV types 2 and 3 DNA in porcine samples.

Species: Porcine

Validated Specimens: Serum, tissue homogenate supernatants, lung homogenates, oral fluid, feces, and fecal swabs (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: \$30 per sample

Turn Around Time: 5 business days

Testing Schedule: Tuesdays and Fridays

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

- Reportable range: Positive Ct \leq 38; Suspect Ct 38.01 – 39.99; Negative Ct $>$ 40
- PCV2: 0.032 TCID₅₀/mL (obtained from a viral isolate)
- PCV3: 20 viral copies/ μ L (obtained from a synthetic sequence)

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

- Within-assay
 - PCV2: 1.1%
 - PCV3: 0.3%
- Between-assay
 - PCV2: 4.5%
 - PCV3: 2.7%

Analytical Specificity:

- 100% (assay was negative when tested for 44 other viruses and bacteria)



Diagnostic Sensitivity and Specificity*:

- | | |
|--------------------------|--------------------------|
| - Diagnostic Sensitivity | - Diagnostic Specificity |
| PCV2: 96% | PCV2: 95% |
| PCV3: 93% | PCV3: 95% |

*Diagnostic sensitivity (DSe) and diagnostic specificity (DSp) were determined by comparing 162 samples (across various sample types) to previously used real-time PCR assays. PCV2 was compared to an in-house assay using the Life Technologies Path-ID Multiplex One-Step RT-PCR kit and PCV3 was compared to an in-house assay using the QuantaBio PerfeCta Multiplex qPCR ToughMix Low ROX kit. Neither of the previously used PCV2 and PCV3 assays provide a true “gold-standard” for comparisons, thus there is inherent error in the simple DSe and DSp calculations performed. *The listed DSe and DSp are relative to the previous, imperfect, PCR assays.*

Of the 324 results obtained (162 for PCV2 and 162 for PCV3), 18 (6%) showed discrepant results when compared to the previous PCR assays. In 8 of these 18 cases, Ct values over 38 were involved, and consistent agreement is not expected in that range. The 10 remaining cases (all with Ct values between 34 and 38), showed a mix of “false negatives” and “false positives” indicating that in some cases the new assay was able to detect virus while the old assay(s) were not.

The kit manufacturer, QIAGEN, also performed an independent validation of the assay and found DSe of 99% and DSp of 100% after testing 243 samples of various types.

Note: The Universal PCV test offered by the UMN VDL detects PCV 1 and 2 (but will not differentiate between 1 and 2), while the new duplex detects PCV 2 and 3 (and differentiates between the two types).