



Porcine Sapelovirus / Teschovirus Real-Time PCR Duplex 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 Sapelovirus (PSV) RNA and Porcine Teschovirus (PTV) RNA in porcine samples.

Validated Specimens: Spinal cord, brain, serum, tissue homogenate, intestines, and feces (other specimen types may be submitted but results will be reported with a disclaimer stating those sample types have not been validated in our lab).

Cost: \$45 per sample

Turn Around Time: 5 business days

Testing Schedule: Fridays

Analytical Sensitivity (the lowest concentration detectable with this assay, as determined by repeatedly testing (n=3) a 1:10 dilution series using viral isolates, with a synthetic RNA target used for the standard curve comparison):

- Reportable range: Positive Ct \leq 37; Suspect Ct 37.01 – 39.99; Negative Ct $>$ 40
- PSV: 20 viral copies/ μ L (obtained from a viral isolate)
- PTV: 20 viral copies/ μ L (obtained from a viral isolate)

Assay Repeatability (aka precision, or how repeatable the data are; presented as the coefficient of determination, or CV %):

- Within-assay (tested as 5 replicates each of 3 isolate dilution levels for each pathogen in a single assay; acceptable CV is $<$ 15%)
 - o PSV: 1.4%
 - o PTV: 1.0%
- Between-assay (3 isolate dilution levels were tested in 10 independent assays over 10 days; acceptable CV is $<$ 20%)
 - o PSV: 5.6%
 - o PTV: 6.4%

Analytical Specificity (a panel of 52 other known bacteria and viruses were tested with the Sapelovirus/Teschovirus PCR to test for cross-reactivity; 0% cross-reactivity, or 100% specificity is expected):

- 100% analytical specificity (the assay did not cross-react with any of the 52 other viruses and bacteria tested)



Diagnostic Sensitivity and Specificity:

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|--------------------------|---------------------------|
| - Diagnostic Sensitivity | - Diagnostic Specificity* |
| PSV: 100% | PSV: 63% |
| PTV: 100% | PTV: 88% |

*Diagnostic specificity (DSp) was calculated by comparing the new real-time PCR (qPCR) to a conventional, gel-based, PCR. Because the qPCR had a better analytical sensitivity (limit-of-detection), it was able to detect more positive samples than the conventional PCR could, especially at Ct values above 28. These discrepant samples were categorized as “false positives” though we suspect they are true positives. A set of 20 samples were sequenced, using Illumina MiSeq version 3 for next-gen-sequencing, to determine true PTV/PSV status. These 20 samples included 19 samples previously tested positive and 1 sample previously tested negative by the real-time PCR. Of the 19 positive samples, 2 had previously shown discrepant results (positive by qPCR but negative by conventional PCR) but were shown to be true positives after sequencing and assembly. All of the 20 samples sequenced matched the qPCR results, suggesting the true DSp of this assay is likely near 100% for PTV and PSV in our set of clinical samples.