SPV/RhPV QPCR Protocol

Materials needed:
Taqman Fast Advanced Master Mix (LifeTechnologies catalog # 4444557)

Assay specific primers and probe (see below) and DNA standard

DEPC water

QPCR reaction plate

QPCR Reaction Set-up
1. Clean work space thoroughly before beginning QPCR set-up
2. Prepare the following master mix according to the chart, making enough mix for each sample and standard to be tested in duplicate:

Reagent	**Stock concentration**	**Volume for 20 ul reaction**	**40 x reaction mix**
Forward primer	10 μM	1 μl	40 μl
Reverse primer	10 μM	1 μl	40 μl
Probe	10 μM	0.4 μl	16 μl
Taqman Fast Advanced MM	2x	10 μl	400 μl
DEPC water	-	2.6 μl	104 μl
Sample DNA	-	5 ul	-

3. Prepare a 1:10 dilution series of the DNA standard, as detailed in the CMV_standard_ preparation protocol
4. Aliquot 15 ul master mix to each well that will be used in the reaction plate
5. Add 5 ul of the standards and negative control to the appropriate wells
6. Add 5 ul of each sample to the appropriate wells
7. Seal plate and spin down briefly before running on QPCR instrument
8. Run the following cycling conditions on the QPCR instrument

Note: These conditions have been tested/optimized on LightCycler plate-based instruments. It is possible they may need to be adjusted for other instruments)

Step	Number of cycles	Temperature	Time	Notes
Activation	1	95°C	5 minutes	
Amplification	50	95°C 60°C 72°C	15 seconds 1 minute 1 second	ramp rate = 3°C/ second, single acquisition at 72°C
Cool	1	40°C	30 seconds	

SPV QPCR assay reagents:
Note: this assay targets the 3' region of the VP2 gene of SPV. All primer and probe sequences are listed from 5'-3'.

SPV_3VP2_forward:
GAGCACGCTAACTGCTTGTTG

SPV_3VP2_reverse:
GCTGTTAGTGGTGGGAATGTG

SPV_3VP2_probe:
6-fam-ACAGGGTCTAGCATTAGCACAGCC-BHQ1

RhPV QPCR assay reagents:
Note: this assay targets the VP2 gene. All primer and probe sequences are listed from 5'-3'.

RhPV_forward:
GGAACGGGTAGCAGCATTAGT

RhPV_reverse:
TGAGAGACACCTTCTAGAGAC

RhPV_probe:
6-fam-ATCAGTTTCCCTCAGTGCAAGCCG-BHQ1