Techne® qPCR test

Slow Bee Paralysis virus

Polyprotein gene

150 tests

For general laboratory and research use only

Introduction to Slow Bee Paralysis virus

Discovered in 1974 in England, Slow Bee Paralysis Virus (SBPV) is characterised by the paralysis of the front two pairs of legs of adult bees Apis mellifera, a few days before dying, which occurs approximately 10 days after infection by the virus.

The virus tends to accumulates in the head, jaw and saliva glands as well as the digestive tract and is less prevalent in the hindlegs of the bee. 362 particles of the virus are enough to kill half the infected host. (LD50)

The SBPV genome is approx.. 9.5kb long with one ORF containing 2964 amino acids. Two strains labelled ‘Rothamsted’ and ‘Harpenden’ and are approx. 83% identical in terms of sequence and 94% homologous in terms of amino acids. Most of the differences are C-U or A-G transitions which are common to RNA viruses.

The SBPV viron is a 30nm icosahedral (20 sided) particle with an RNA genome and 3 major capsid proteins. It is similar to picorna-like mite transferred viruses but is relatively rare. Like Deformed wing virus, V.Destructor virus 1 and sachrood virus, the genome is arranged with the structural proteins being encoded in the N-terminal ORF and other proteins in the C-terminal area.

The virus is associated with, and transmitted to adult bees and pupae by Varroa destructor mites. Despite this association, SBPV is rarely detected in bee colonies even if Varroa is detected.

SBPV can is asymptomatic in larvae and pupae, but can be detected at this stage.
Ultimately SBPV can cause colony collapse disorder and result in total destruction of the hive.

Because SBPV is highly virulent, killing the host bee rapidly before significant replication, it is relatively rare in colonies even those which have a strong V.destructor mite presence.

Specificity

The Techne® qPCR Kit for Slow Bee Paralysis virus (SBPV) genomes is designed for the in vitro quantification of SBPV genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the SBPV genome.
The primers and probe sequences in this kit have 100% homology with a broad range of SBPV sequences based on a comprehensive bioinformatics analysis.

The kit has been developed to detect all subtypes of Slow Bee Paralysis Virus (SBPV) and will detect both the Rothamsted and Harpenden strains.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to technehelp@bibby-scientific.com and our bioinformatics team will answer your question.

Kit Contents

- **SBPV specific primer/probe mix (150 reactions BROWN)**
  FAM labelled

- **SBPV positive control template (for Standard curve RED)**

- **Internal extraction control primer/probe mix (150 reactions BROWN)**
  VIC labelled as standard

- **Internal extraction control RNA (150 reactions BLUE)**

- **Endogenous control primer/probe mix (150 reactions BROWN)**
  FAM labelled

- **SBPV/Internal extraction control/endogenous control RT primer mix (150 reactions GREEN)**
  Required for two step protocol only

- **RNAse/DNAse free water (WHITE)**
  for resuspension of primer/probe mixes and internal extraction control RNA

- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

RNA extraction kit
This kit is rdesigned to work well with all processes that yield high quality RNA with minimal PCR inhibitors.

Lyophlised OneStep 2x qRT-PCR MasterMix
Contains complete one step qRT-PCR MasterMix

Pipettors and Tips

Vortex and centrifuge

1.5 ml tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Techne does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNAse/DNAse free water.

Dynamic range of test

Under optimal PCR conditions Techne SBPV detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

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Principles of the test

Real-time PCR

A SBPV specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the SBPV cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template.
This can be used to generate a standard curve of SBPV copy number / CT value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target SBPV gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNAse/DNAse free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Internal RNA extraction control

When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and real-time PCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

A separate RT primer mix and a real-time PCR primer/probe mix are supplied with this kit to detect the exogenous RNA using real-time PCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the SBPV target cDNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a CT value of 28+/-3 depending on the level of sample dilution.

Endogenous control

To confirm extraction of a valid biological template, a primer and probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel and it is NOT therefore possible to perform a multiplex with the SBPV primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Carry-over prevention using UNG (unsuitable for onestep procedure and optional for two step procedure)

Carry over contamination between PCR reactions can be prevented by including uracil-N-

glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. Techne recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step.

Reconstitution Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Reconstitute the kit components in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
SBPV primer/probe mix (BROWN)	165 µl
Internal extraction control primer/probe mix (BROWN)	165 µl
RT primer mix (GREEN)	165 µl
Endogenous control primer/probe mix (BROWN)	165 µl
Pre-PCR heat-sealed foil	
Internal extraction control RNA (BLUE)	600 µl

3. **Reconstitute the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex this tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Positive Control Template (RED) *	500 µl

*** This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.**

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.**

2. **Complete RNA extraction according to the manufacturers protocols.**

One Step RT-PCR detection protocol

A one step approach combining the reverse transcription and amplification in a single closed tube is the preferred method.

For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the One Step amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each RNA sample prepare a reaction mix according to the table below: Include sufficient reactions for positive and negative controls.**

Component	Volume
OneStep 2x qRT-PCR MasterMix	10 µl
SBPV primer/probe mix (BROWN)	1 µl
Internal extraction control primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	3 µl
Final Volume	**15 µl**

2. **For each RNA sample prepare an endogenous control reaction according to the table below (optional):**
This control reaction will provide crucial information regarding the quality of the biological sample.

Component	Volume
OneStep 2x qRT-PCR MasterMix	10 µl
Endogenous control primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

3. **Pipette 15µl of these mixes into each well according to your real-time PCR experimental plate set up.**

4. **Pipette 5µl of RNA template into each well, according to your experimental plate set up.**
For negative control wells use 5µl of RNAse/DNAse free water. The final volume in each well is 20µl.

5. **If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:**

Component	Volume
OneStep 2x qRT-PCR MasterMix	10 µl
SBPV primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

6. **Preparation of standard curve dilution series.**

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10 µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2×10^5 per µl
Tube 2	2×10^4 per µl
Tube 3	2×10^3 per µl
Tube 4	2×10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. **Pipette 5µl of standard template into each well for the standard curve according to your plate set-up**
The final volume in each well is 20µl.

One Step Amplification Protocol

Amplification conditions using OneStep 2x RT-qPCR MasterMix.

	Step	Time	Temp
	Reverse Transcription	10 mins	42 °C
	Enzyme activation	2 mins	95 °C
50 Cycles	Denaturation	10 secs	95 °C
	DATA COLLECTION *	60 secs	60 °C

* Fluorogenic data should be collected during this step through the FAM and VIC channels

Interpretation of Results

Target	Internal control	Negative control	Positive control	Interpretation
+ive	+ive	-ive	+ive	**+ive**
+ive	-ive	-ive	+ive	**+ive**
-ive	+ive	-ive	+ive	**-ive**
-ive	-ive	-ive	-ive	**Experiment fail**
+ive	+ive	+ive	+ive	**Experiment fail**

Internal PCR control

The CT value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. CT values of 28±3 are within the normal range. When amplifying a SBPV sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Endogenous control

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.