1- step HLVd RT-qPCR Validation

1. To validate the efficiency and specifications of 1-step RT-qPCR for HLVD detection, we ran the one-step RT-qPCR performance using the data procedures. The positive control was used to detect amplification efficiency, and negative control is used to amplification specification. To validate the efficiency, the positive control was diluted in 10-fold dilution from 10,000fg/ µl to 0.001fg/ µl as Table 1.

Table 1 Positive Control concentration

Dilution	pg/µl	fg/µl
10^1	10	10000
10^0	1	1000
10^-1	0.1	100
10^-2	0.01	10
10^-3	0.001	1
10^-4	0.0001	0.1
10^-5	0.00001	0.01
10^-6	0.000001	0.001

2. Set up RT-qPCR mix for each reaction described as the data sheet. The detail was listed as Table 2.

Table 2 RT-qPCR mix preparation

Components	Volume (ul) /reaction
4x RT-PCR Mix	5
primer-probe	2
4xRT-PCR	5
DEPC-water	6
Positive control /negative control	2
Final vol.	20

3. 1-step RT-qPCR reaction and amplification was made as Table 3.

Table3 RT-qPCR condition same as data sheet

Step	Temperature	Duration	Cycle(s)
1	50°C	20 mins	1
2	95°C	1 min	1
3	95°C	15 secs	
	60°C	60 secs	40

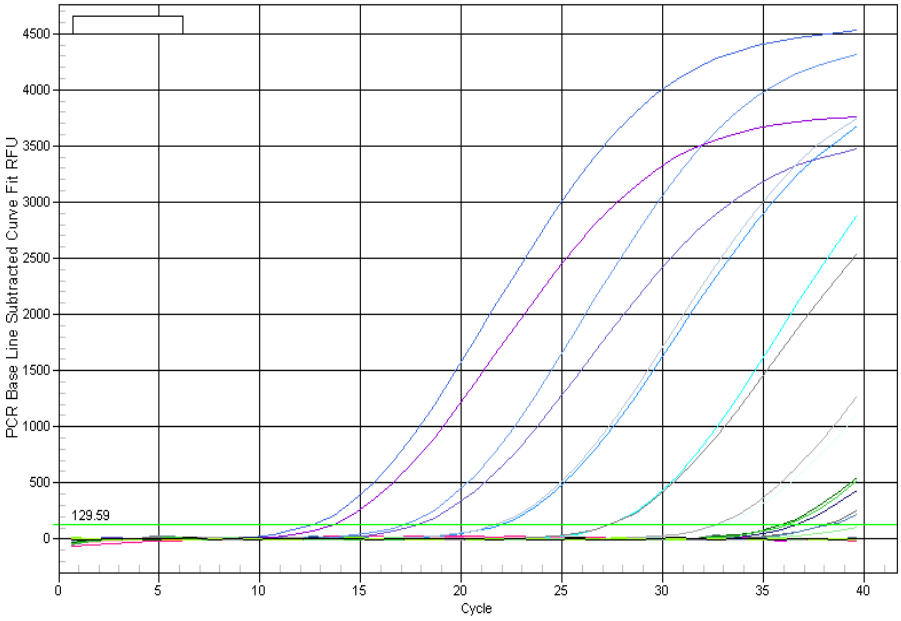
4. Results:

RT-qPCR has very high sensitivity and accuracy for HLVD detection. The data was shown as Table 4 and Figure 1.

Table4 RT-qPCR amplification

Positive Control	pg/ µl	fg/ µl	Ct Value for Fam-Positive		Ct value for Hex-internal control	
10^1	10		12.64	13.54	16.97	17.94
10^0	1	1000	17.14	17.85	18.04	17.95
10^-1	0.1	100	21.92	21.72	17.06	16.79
10^-2	0.01	10	27.31	27.27	17.33	16.98
10^-3	0.001	1	32.58	32.88	17.53	17.09
10^-4	0.0001	0.1	35.9	38.08	17.21	17.23
10^-5	0.00001	0.01	36.16	36.62	17.67	17.58
10^-6	0.000001	0.001	38.36	no	17.7	17.82
Negative	0	0	no	no	18.64	20.12

Fig 1 RT-qPCR amplification with Positive control at different concentration



From Table 4 and Figure, the data demonstrate the 1-step HLVd RT-qPCR had a high efficiency, accuracy for HLVd. We used this kit to successfully identified if the cannabis leaves were infected by the HLVd (data not shown here).

Conclusion: 1-step HLVd RT-qPCR Kit (TBS42015) has a high fidelity and efficiency for HLVd detection.