Sensitivity and Specificity of Norgen's HLVd TaqMan RT-PCR Kit

Application Note 98

Keywords

+ HLVd
+ Hop latent
+ Viroid
+ Agriculture
+ Cannabis
+ Hops
+ Hemp
+ Plant RNA
+ Dudding
+ RT-qPCR
+ Plant disease
+ Biosecurity management
+ Infection

INTRODUCTION

Humulus lupulus, commonly referred to as the hop plant, is recognized globally as a key ingredient in beer. Hop plants belong to the Cannbinaceae taxonomic family, which also contains hemp and marijuana. A mounting concern which has both economic and agricultural impacts is the infection of Hop Latent Viroid disease, also known as HLVd, observed in the plants of this family. Common symptoms of infection include stunted growth, necrosis of leaves, as well as yellowing and curling leaves. Most notably in hops, infection significantly reduces the alpha-acid content - the key component which confers taste and aroma. In cannabis plants, infection can severely reduce trichome yield and potency. Both can have devastating economic implications on their respective industries.

Symptoms are typically not visible in infected plants until they reach maturity, hence the name hop "latent" viroid. Consequently, early molecular detection is key to prevent spread throughout crops, as current remediation methods involve destroying all infected plants.

Norgen Biotek Corp. provides an effective and optimized kit for early detection of HLVd. Hop latent viroid consists of a piece of small RNA, only ~250 base pairs in length. General extraction methods using silica and magnetic beads have been shown to exclude small base pair fragments, however, Norgen's silicon carbide extraction method shows no such bias towards molecular weight or G-C content. Here we highlight the specificity and sensitivity of Norgen's HLVd detection kit (Cat. TM38700, TM38710).

MATERIALS AND METHODS

In silico Analysis of Reactivity of Primers and Probes.

Sequence alignment of Norgen's HLVd primers with known HLVd sequences via BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was conducted to determine the homology of primers relative to known viroid sequences.

Quantification and Preparation of HLV Transcript.

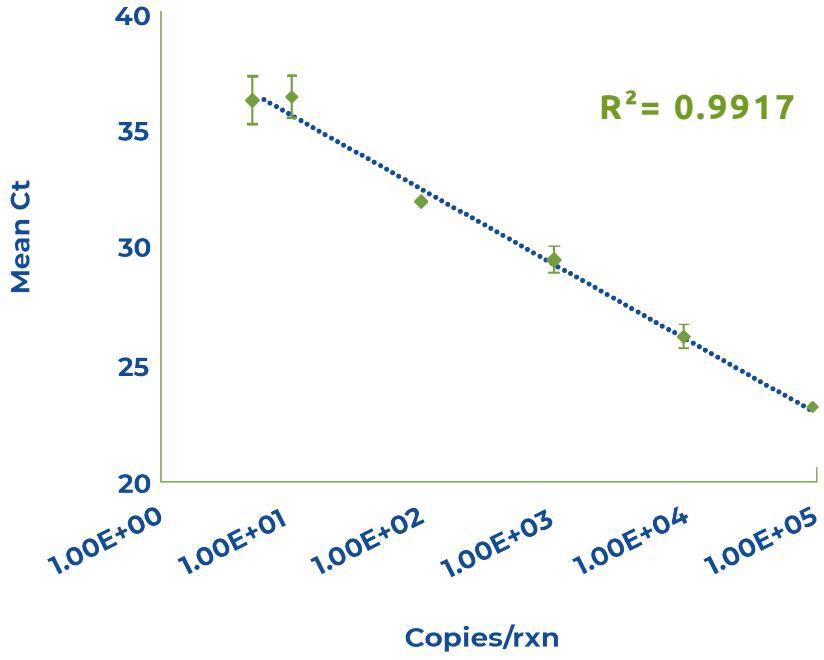
Based on the concentration of positive control quantified by The Hospital for Sick Children via digital droplet PCR (RT-ddPCR), a dilution series was prepared. This range contained 0.125 to 12,500 transcript copies/µL, which results in 1 to 10,000 copies per PCR reaction. RT-PCR reaction was prepared according to that outlined in Norgen's kit protocol (cat. TM38700, TM28710). The input for each RT-PCR reaction was 8 µL of each dilution, which was loaded in triplicate. A no template control (NTC) was also included in duplicate.

RESULTS & DISCUSSION

Limit of Detection of Norgen's HLVd Detection Kit.

To determine the limit of detection for Norgen's HLVd detection kit, a serial dilution using quantified positive control was prepared according to the aforementioned range. RT-PCR was conducted according to the instructions outlined in the kit and the limit of detection observed was 5 copies of RNA transcript per PCR reaction (Table 1). A standard curve was produced using the standard RNA quantified by ddPCR (Figure 1) with an $R^2 = 0.9917$ which can be used to quantitatively measure the amount of copies present in a given sample.

FIGURE 1



Mean Ct. scatter plot of HLVd positive control transcript (1-10^5 copies) with linear detection between 10 and 10^5 copies ($R^2 = 0.9917$)

TABLE 1

HLVd RT-qPCR data

Ct. values and associated mean and standard deviation corresponding to the number of HLVd copies per reaction

Copies/rxn	Ct. Values	Mean Ct.	STDEV Ct.
100000 (1.00E+05)	23.65	23.33	0.29
	23.10		
	23.23		
10000 (1.00E+04)	25.80	26.21	0.46
	26.13		
	26.70		
1000 (1.00E+03)	29.29	29.48	0.50
	29.11		
	30.05		
100 (1.00E+02)	31.79	31.86	0.08
	31.95		
	31.84		
10 (1.00E+01)	37.29	36.37	0.88
	36.29		
	35.53		
5 (5.00E+00)	37.24	36.28	1.01
	36.36		
	35.23		
1 (1.00E+00)	39.45	N/A	N/A
	N/A		
	N/A		
NTC	N/A	N/A	N/A
	N/A		
	-		

Specificity of Primers and Probes in Norgen's HLVd Detection Kits

Norgen's HLVd detection kits are able to detect with a high degree of confidence the following HLVd variants referenced in Table 2 (p.4) without concern for cross-reactivity between variants

Application Note 98

For more information about HLVd and its effects on crops visit

norgenbiotek.com/viroid-detection

TABLE 2

HLVd Kit Cross-Reactivity Against Regional Variants Assessed

Organism	Country	Accession	% Homolgy of qHLVd-F	% Homology of qHLVd-R	% Homology of qHLVd-P
HLVd-C1	**USA**	MK876285.1	100%	100%	100%
HLVd-GVdC_ HLVd01	**Belgium**	KT600318.1	100%	100%	<80%
HLVd-RefSeq	**Germany**	NC_003611.1	100%	100%	100%
HLVd-T92	**Czech Republic**	AJ290410.1	95%	100%	100%
HLVd-Y7	**China**	EF613192.1	100%	100%	96%

When HLVd detection kit components are assessed against other potential pathogens which may be present in a given plant sample, the potential for cross-reactivity is extremely low due to the lack of compatible sequences observed in primers and probes (Table 3).

TABLE 3

HLVd Kit Cross-Reactivity Against Other Major Pathogens

		HLVD Taqman RT-PCR Kit (Cat. TM38700)		HLVD Taqman Probe/Primer and Control Set (Cat. TM38710)	
Organism	Accession	% Homology of HLVd-F	% Homology of HLVd-R	% Homology of qHLVd-F	% Homology of qHLVd-R
Apple mosaic virus	12319	0%	0%	0%	0%
Arabis mosaic virus	12271	0%	0%	0%	0%
Citrus bark cracking viroid	NC_003539.1	0%	0%	0%	0%
Coconut cadang-cadang viroid	NC_001462.1	0%	0%	0%	0%
Coconut tinangaja viroid	NC_001471.1	0%	0%	0%	0%
Columnea latent viroid	NC_003538.1	0%	0%	0%	0%
Hop latent virus	104263	0%	0%	0%	0%
American Hop Latent virus	1177630	0%	0%	0%	0%
Hop mosaic virus	142843	0%	0%	0%	0%
Hop stunt viroid	NC_001351.1	0%	0%	0%	0%
Potato spindle tuber viroid	NC_002030.1	0%	0%	0%	0%
Tomato chlorotic dwarf viroid	NC_000885.1	0%	0%	0%	0%
Alfalfa mosaic virus	NC_002025.1 NC_001495.1 1NC_002024.2	0%	0%	0%	0%
Cucumber mosaic virus	12305	0%	0%	0%	0%
Tobacco mosaic virus	12242	0%	0%	0%	0%

CONCLUSIONS

Norgen's HLVd Taqman Detection Kits (TM38700, TM38710) are able to reliably detect as few as 5 viral copies per RT-PCR reaction. Therefore, to ensure accuracy of the assay a minimum of 125 copies/µL of viroid genome in sample RNA must be present.

Furthermore, the following kit is expected to successfully detect HLVd from the regions of the USA, Belgium, China, Germany, and the Czech Republic with a high degree of confidence without concern for cross-reactivity to other major pathogens assessed herein.

Related Products	Reasearch Use
HLVd TaqMan RT-PCR Kit	TM38700
HLVd TaqMan Probe/Primer and Control Set	TM38710
HLVd End-Point RT-PCR Kit	EP38700
HLVd Primer and Control Set	EP38710

Related Blogs

HLVd: The Culprit of Cannabis Crop Failure & Dudding

Viroids: An Agriculturist's Worst Nightmare

To read related blogs go to:

norgenbiotek.com/norblog

Norgen Biotek Corp.
3430 Schmon Parkway,
Thorold, ON, Canada, L2V 4Y6

E info@norgenbiotek.com
T 1 866 NORGENB
norgenbiotek.com