Validation of TUMIGlow™ Platform for Hop Latent Viroid Detection

Background

Hop Latent Viroid (HLVd) is a small, circular, infectious agent. As opposed to viruses and other pathogens, HLVd lacks an outside protein and is only composed of genetic material (RNA). Viroids generally spread via mechanical transmission on unsterilized cutting tools and equipment. HLVd has been detected in most geographical locations around the world and identified in cannabis plants throughout the United States, Europe and Canada. Common symptoms of HLVd in cannabis plants include stunted growth, brittle stems, leaf malformation and reduced flower mass. However, plants may initially appear asymptomatic or with subtle symptoms making detection by eye difficult. For more detailed information on HLVd biology and transmission see our website: https://www.tumigenomics.com/hop-latent-viroid-information

The most reliable way to determine if HLVd is spreading through a cannabis crop is by performing regular screening of plants using a molecular (nucleic acid amplification) test. We recommend screening mother plants every 4-6 weeks or at least twice in the productive lifetime of each mother.

Test Description

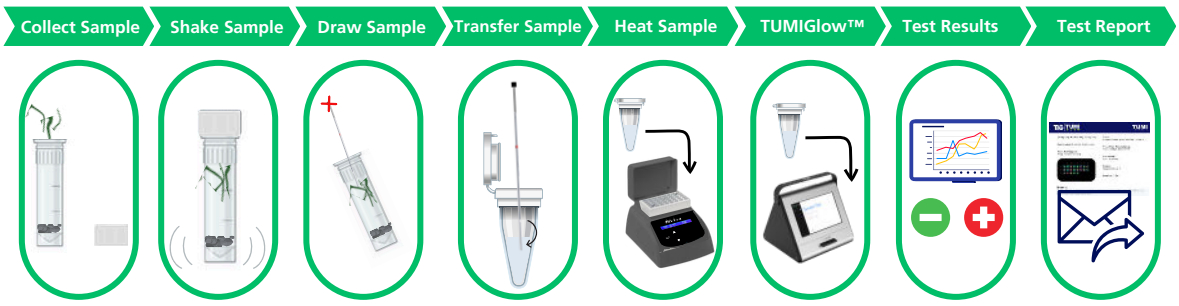
TUMI Genomics' on-site hop latent viroid (HLVd) assay, TUMIGlow, detects the presence of HLVd infection in cannabis/hemp plant tissue. The TUMIGlow-HLVd test is based on Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) technology. However, several key changes to standard RT-LAMP amplification allow the TUMIGlow technology to be dramatically more sensitive and specific, while drastically decreasing both false positive and false negative results.

Key Takeaways:

- **The TUMIGlow-HLVd assay is *as sensitive as a PCR,*** while still being simple, flexible, and rapid.
- **TUMIGlow-HLVd tests don't require technical expertise**, additional equipment or purchase of extra consumables, like pipette tips.
- **The TUMIGlow-HLVd assay includes an internal control** that detects a cannabis RNA sequence so negative test results give a signal that is distinct from failed test results. This feature is critical to be confident in HLVd negative tests.

Analysis of TUMIGlow test results is fast, simple, and reliable because they are interpreted by a specialized device and software. This feature removes confusion or uncertainty when judging the infection status of your plants.

Testing Process: The TUMIGlow-HLVd test is easy to perform. Root tissue is added to a sample collection tube and mixed for 3-5 seconds. A provided transfer stick is used to move a portion of the sample into the test. Tests are incubated for 90 minutes, cooled and results are interpreted by the TUMIGlow analysis device.



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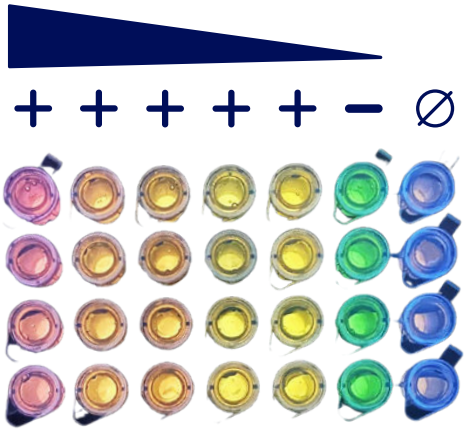
Result Analysis and Tracking: TUMIGlow-HLVd tests are analyzed by the Glow Device. The Glow Device software can determine the results of 48 TUMIGlow-HLVd tests in less than a minute, allowing hundreds of samples to be tested in a day. Results are displayed in a spreadsheet like format and select results can be exported into a document to be shared internally or with customers looking to purchase your genetics. The Glow Device dashboard allows visual tracking of plant health throughout a facility(s) by room, plant stage, or test user.

TUMIGlow™ Device

The TUMIGlow Device operates using a touch-screen and utilizes a wi-fi connection to store and share results.

TUMIGlow - HLVd Tests

HLVd Viroid Level



TUMIGlow - HLVd Test Report

The TUMIGlow generates digital reports than can be downloaded or shared with other parties.

1. The top portion of the report indexes all the information about the test run.
2. The instrument's internal mechanics record a photo of the sample results after each run.
3. A section to record notes and share results and cultivation decisions.
4. The results are recorded in a table that allows for manual input regarding the sample name, strain, and any notes.

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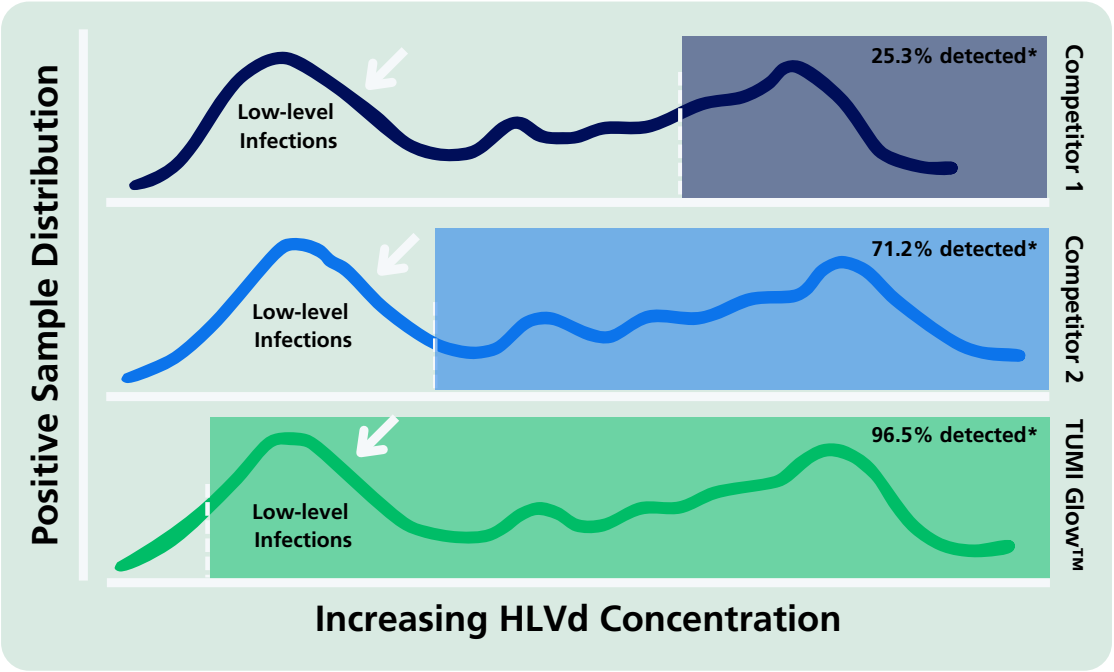
Validation Summary

Sensitivity: TUMIGlow-HLVd assay was compared to TaqMan qRT-PCR on a range of sample concentrations. The TUMIGlow-HLVd detected down to 4 HLVd copies per microliter with >95% accuracy, which equals a cycle threshold of 33.2 (CT=33.2), making this onsite test as sensitive as TaqMan qRT-PCR and 100X more sensitive than the most sensitive hop latent viroid field test on the market.



Inclusivity: The primers used in the TUMIGlow-HLVd tests were carefully designed to allow detection of known HLVd sequence variants. Based on Insilco analysis, TUMIGlow-HLVd assay can detect >95% of HLVd sub-species. Analysis on actual customer samples indicates 100% detection of samples originating from diverse geographical locations including: Canada, United Kingdom, Switzerland, Nederland, Italy, Austria, Portugal, Greece and Thailand.

Accuracy: The TUMIGlow-HLVd test performs with 99.1% accuracy compared to PCR. The TUMIGlow test detects 100% of HLVd(+) samples down to ~20 viroid copies/µL. No false negative results were obtained within this range and no false positive results were observed throughout the entire experiment.

Specificity: Comparison of TUMIGlow-HLVd primers to the genome sequences of 48 known cannabis pathogens and the cannabis DNA sequence showed no cross-reactivity. Wet lab testing of common cannabis root pathogens such as Fusarium and Pythium showed no interference or cross-reactivity with the TUMIGlow-HLVd test, indicating the test is very specific for hop latent viroid and contaminating pathogens do not affect the results.

Flexibility: The TUMIGlow-HLVd field test was subjected to a variety of conditions to determine the flexibility of the test when deviating from the instructions. These experiments showed that the TUMIGlow-HLVd test still functions correctly even when there were: errors in test volume, errors in incubation time, a delay in result analysis, or errors in tissue amount. These studies indicate that TUMIGlow-HLVd tests are robust when used by inexperienced or non-professional users, which is critical for a field test.

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Performance Evaluation

A performance evaluation consists of experiments performed by TUMI Genomics to determine the technical (in lab) limits and characteristics of the assay.

Analytical Sensitivity

Limit of Detection

Sensitivity, Limit of Detection (LOD): A limit of detection study determines the sensitivity of a test. The limit of detection is defined as the lowest concentration of pathogen where the test can still detect 95% of the true positive samples. A limit of detection can help you understand how well a test can find low-level/early infections.

To determine the limit of detection (LoD) of the TUMI Genomics TUMIGlow-HLVd assay we spiked negative plant extract with decreasing amount of HLVd sequence for twenty replicates of each tested concentration as shown in the table below. These studies indicated that the assay can reliably detect down to 4 viroid copies per microliter.

Table shows the results from studies performed to determine the LoD of TUMI Genomics HLVd qRT-PCR assay. The identified LoD is indicated in blue.

HLVd Concentration in Sample	Fraction Positive TUMIGlow	Percent Detection Success	Average Cycle Threshold HLVd Target (PCR)	Average Cycle Threshold Plant Target
4.9 X 10^5 copies/µL	20/20	100%	16.15	28.27
5.8 X10^4 copies/µL	20/20	100%	19.54	28.09
7.4 X10^3 copies/µL	20/20	100%	22.44	28.22
826 copies/µL	20/20	100%	25.56	28.18
140 copies/µL	20/20	100%	27.9	28.33
34 copies/µL	20/20	100%	29.8	28.16
11 copies/µL	20/20	100%	31.6	27.29
6 copies/µL	20/20	100%	32.4	29.30
4 copies/µL	20/20	100%	33.2	27.98
2 copies/µL	16/20	80%	NaN	26.78
1 copies/µL	10/20	50%	NaN	28.76
0 copies/µL	0/24	0%	NaN	29.35

Inclusivity

An inclusivity analysis determines how well a test can detect different known sequence variants of a given pathogen. An acceptable inclusivity analysis should show that an assay has been designed in a way that can reasonability detect all, or the vast majority, of known variants of the target pathogen.

An in-silico inclusivity analysis was performed by aligning all primer sequences targeting the hop latent viroid genome in the TUMIGlow-HLVd assay against hop latent viroid variants deposited within GenBank. A total of 155 genomes were in the repository representing 116 unique sequences. To focus on well represented sequence variations, only positions where 5% or more of the known sequences showed a mismatch relative to the consensus sequence were considered.

Of the 155 genomes, 149 (96%) had either no primer sequences showing well-represented mismatches or a mismatch occurred in only a single position in any given primer. These genomes are predicted to be readily detected by the TUMIGlow-HLVd assay.

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Of the 6 remaining HLVd genomes, 4 showed two mismatches in a single primer and 2 showed three mismatches in a single primer. No HLVd genomes showed two or more mismatches in multiple primer sequences. Given the heterogenous nature of viroid genome sequences within an individual plant (Adkar-Purushothama et. al, 2020) the TUMIGlow-HLVd assay is predicted to detect the vast majority of the HLVd sequence pools in an infected cannabis plant.

Table shows the number of annotated HLVd genomes showing well-represented mismatches with regions targeted by the primer used in the TUMIGlow-HLVd assay.

Number of Mismatches	HLVd Genomes	Percent Homology
No Mismatches	95 (66%)	100%
One or less total mismatches	127 (82%)	99.6%
Two or less mismatches	148 (96%)	99.4%
Three of less mismatches	154 (99%)	99.1%

Because limited published evidence exists regarding the frequency of sequence variants in natural infections, we further validated the specificity of TUMIGlow-HLVd assay by testing HLVd + samples (as determined by TaqMan-RTPCR) from nationally and internationally diverse locations. 146 samples collected by cultivators across the USA and globally were tested. In all tested samples, the TUMIGlow-HLVd assay was able to detect the presence of an infection regardless of geographical origin.

Table indicates the percent accuracy of detection of HLVd+ samples from the indicated international locations.

Location of Sample Collected	HLVd+ Samples by PCR	Fraction HLVd+ (TUMIGlow)	HLVd CT Range (PCR)	Viroid Load Range (HLVd copies/µL) High	Low	% Accuracy TUMIGlow
Austria	5	5/5	24.2 - 32.5	12603.1	2122.8	100%
Canada	6	6/6	23.4 - 33.4	3640.3	5.8	100%
Greece	6	6/6	21.7 - 32.9	12603.1	4.4	100%
Italy	7	7/7	19.7 - 33.2	44889.6	3.6	100%
Netherlands	7	7/7	21.5 - 31.5	14018.7	8.62	100%
Portugal	8	8/8	22.6 - 32.8	6331.8	4.58	100%
Switzerland	5	5/5	19.1 - 32.5	45081.4	5.6	100%
Thailand	6	6/6	23.6 - 33.1	3048.5	3.76	100%
UK	12	12/12	20.1 - 34.1	37326.3	1.82	100%

Table indicates the percent accuracy of detection of HLVd+ samples from the indicated locations within the United States. **Below the limit of detection of the TUMIGlow-HLVd assay

Location of Sample Collected	HLVd+ Samples by PCR	Fraction HLVd+ (TUMIGlow)	HLVd CT Range (PCR)	Viroid Load Range (HLVd copies/µL) High	Low	% Accuracy TUMIGlow
West (CA, OR, CO, MT, NV, UT)	17	17/17	17.0 - 31.0	341650.6	16.7	100%
Mid-West (MI, OH, IL, IN, MO)	10	10/10	20.1 - 31.1	37326.3	15.8	100%
South West (AZ, NM, OK, TX)	9	9/9	19.8 - 28.5	46511.0	95.5	100%
South East (FL, MS, NC, KY, VA, TN)	12	11/12	18.0 - 34.3	164490.9	1.6**	92%
North East (PA, NY, ME, MA, NH, MD, CT, VT)	20	20/20	17.3 - 32.3	284087.1	7.3	100%
Alaska, Hawaii	5	5/5	22.3 - 27.5	7889.9	99.8	100%

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Analytical Sensitivity

Cross Reactivity

A cross reactivity analysis determines whether an assay is specific to the target pathogen (i.e. HLVd) versus giving a non-specific signal or throwing a positive result due to the presence of a contaminating pathogen. A specific test should be 100% specific for the target pathogen and less than 80% specific for potential contaminating pathogens (like other viruses or fungal organisms).

In Silico Analysis: Cross-reactivity of the TUMI Genomics' test was evaluated using in silico analysis of primer sequences compared to genome sequences from microorganisms that are commonly found in the roots, stems, and leaves of cannabis, hops, and hemp plants. Genomic sequences for the microorganisms were acquired from the National Center for Biotechnology Information database (NCBI) and the alignments were performed with the Basic local alignment search tool (BLAST). The cannabis sativa reference genome cs10 was used for cross-reactivity analysis against primers and selected microorganisms. For each organism, percent cross-reactivity was determined by dividing the number of nucleotide sequences that matched with the organism by the total number of nucleotides in the HLVd primer/probe set or the internal control cannabis target.

- To adjust for short input sequences and increase the number of detected sequences, the parameter word_size was set to 5.
- Match and mismatch scores were set to 1 and -3, respectively.
- The penalty to create and extend a gap in an alignment was set to 5 and 2, respectively.

Cross-reactivity is defined as greater than 80% similarity between the primer/probes set and any sequence present in the targeted microorganism. As expected, primer probe sequences matched 100% with sequences from the Hop Latent Viroid genome. No cross-reactivity above 80% was found with any other tested microorganism.

The table shows the identity of each tested microorganism and the percent cross-reactivity with the primer/probe sets used to target the HLVd genome and the cannabis genome. Cross-reactivity is shown separately for each primer/probe set.

Organism	Significant Cross-Reactivity?	% Cross Reactivity - HLVd primer/probes	% Cross Reactivity - Cannabis primer/probes
Hop Latent Viroid	NA	100%	30.72%
Cannabis Sativa	NA	35.29%	100%
Cercospora Flagellaris	No Cross Reactivity	74.05%	77.12%
Cladosporium Cladosporioides	No Cross Reactivity	70.89%	73.86%
Phoma XZ068	No Cross Reactivity	70.25	49.67%
Pseudomonas Syringae	No Cross Reactivity	70.25%	75.82%
Berkeleyomyces Basicola	No Cross Reactivity	68.35%	74.51%
Macrophomina Phaseolina	No Cross Reactivity	68.35%	77.78%
Alternaria_alternata	No Cross Reactivity	67.72%	67.97%
Fusarium Oxysporum	No Cross Reactivity	66.46%	74.51%
Phytophthora Citricola	No Cross Reactivity	66.46%	65.36%
Verticillium Albo-atrum	No Cross Reactivity	65.82%	74.51%
Fusarium Solani	No Cross Reactivity	65.19%	66.01%
Rhizoctonia Solani	No Cross Reactivity	64.56%	56.86%
Botrytis Cinerea	No Cross Reactivity	62.03%	65.36%
Pythium Oligandrum	No Cross Reactivity	61.39%	64.71%
Aster yellows witches Broom	No Cross Reactivity	59.49%	61.44%
Pythium Guiyangense	No Cross Reactivity	57.59%	47.06%
Citrus Bark Crackling Viroid	No Cross Reactivity	56.96%	40.52%

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Organism	Significant Cross-Reactivity?	% Cross Reactivity - HLVd primer/probes	% Cross Reactivity - Cannabis primer/probes
Tetranychus Urticae	No Cross Reactivity	55.70%	47.71%
Curvularia Lunata	No Cross Reactivity	55.06%	58.82%
Sclerotinia Sclerotiorum	No Cross Reactivity	55.06%	55.56%
Hop Mosaic Virus	No Cross Reactivity	51.90%	53.59%
Beet Curly Top Virus	No Cross Reactivity	50.00%	50.98%
Tobacco Ring Spot Virus	No Cross Reactivity	50.00%	50.33%
Colletotrichum Fioriniae	No Cross Reactivity	49.37%	46.41%
Potato Virus X	No Cross Reactivity	49.37%	51.63%
Verticillium Dahliae	No Cross Reactivity	49.37%	58.17%
Tomato Ring spot Virus	No Cross Reactivity	48.73%	53.59%
Pythium Insidiosum	No Cross Reactivity	48.10%	44.44%
Potato Virus Y	No Cross Reactivity	47.47%	51.63%
Cucumber Mosaic Virus	No Cross Reactivity	46.84%	52.29%
Xanthomonas Cannabis	No Cross Reactivity	46.20%	40.52%
Tomato Mosaic Virus	No Cross Reactivity	45.57%	51.63%
Arabis Mosaic Virus	No Cross Reactivity	44.30%	47.06%
Alfalfa Mosaic Virus	No Cross Reactivity	43.67%	47.71%
Tobacco Streak Virus	No Cross Reactivity	43.67%	51.63%
Apple Fruit Crinkle Viroid	No Cross Reactivity	42.41%	40.52%
Hop Stunt Viroid	No Cross Reactivity	41.77%	37.25%
Cannabis cryptic virus	No Cross Reactivity	39.87%	51.36%
Pythium Periplocum	No Cross Reactivity	36.71%	38.56%
Fusarium Sambucinum	No Cross Reactivity	34.81%	28.10%
Pythium Aphanidermatum	No Cross Reactivity	29.11%	28.76%
Pythium Brassicum	No Cross Reactivity	29.11%	19.61%
Stemphylium Lycopersici	No Cross Reactivity	29.11%	31.37%
Stemphylium Vesicarium	No Cross Reactivity	28.48%	38.56%
Pseudoperonospora Humuli	No Cross Reactivity	20.89%	25.49%
Pythium Arrhenomanes	No Cross Reactivity	20.25%	16.99%

Wet Lab Analysis

In Silico Analysis: To confirm the specificity of the TUMIGlow-HLVd assay, we conducted a wet lab cross-reactivity/inference assay with cannabis pathogens likely to be encountered during cultivation. No cross-reactivity nor interference resulting from addition of the tested organisms was observed.

Table shows results of cross reactivity and interference analyses using the indicated contaminating pathogens.

Pathogen	Concentration (genome copies/rx)	Successful Tests		Conclusion
Fusarium oxysporum	5.2X10^6	3/3	3/3	No Cross-reactivity/ No Interference
Fusarium solani	1.4X10^6	3/3	3/3	No Cross-reactivity/ No Interference
Fusarium proliferatum	2.2X10^6	3/3	3/3	No Cross-reactivity/ No Interference
Pythium ultimatum	2.7X10^5	3/3	3/3	No Cross-reactivity/ No Interference
Pythium aphanidermatum	8.5X10^5	3/3	3/3	No Cross-reactivity/ No Interference
Pythium dissotocum	2.7X10^5	3/3	3/3	No Cross-reactivity/ No Interference
Pythium myriotylum	2.7X10^5	3/3	3/3	No Cross-reactivity/ No Interference
PhytoPythium Spp.	1.3X10^6	3/3	3/3	No Cross-reactivity/ No Interference
Trichoderma	1.8X10^6	3/3	3/3	No Cross-reactivity/ No Interference

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Interfering Substance Studies

An interfering substance study identifies chemicals and substances likely to be present in a sample that may impair the function of the assay. This analysis helps to determine which treatments and chemicals are safe to use while performing the TUMIGlow-HLVd test and which require caution.

Each tested substance was evaluated at the highest relevant concentration (worst case). Positive samples were created by spiking HLVd positive material in negative root tissue extract at 3X the limit of detection of the test (~12 viroid copies/µL) All substances were tested in triplicate for both HLVd negative and positive samples . Among tested substances, the majority showed no impact on efficacy of the TUMIGlow-HLVd assay. Follow-up experiments shown below were performed on substances that showed some interference.

Table shows the substances tested in the TUMIGlow-HLVd assay and if those substances interfere with an accurate result.

Substance	Concentration	Details	Successful Tests HLVd -	Successful Tests HLVd +	Conclusion
Growing Medium					
Coco Coir	~ 150mg per sample	Added as a coating on outside of wet root tissue	3/3	3/3	No Interference
Soil	~ 150mg per sample	Added directly to sample tube	0/3	0/3	Interference
Rock Wool	~ 50mg per sample (dry)	Added directly to sample tube	0/3	0/3	Interference
Disinfectants					
Chlorox Bleach	20% solution of 7.5% sodium hypochlorite	Fully submerged scissors used to cut root without rinse	0/3	0/3	Interference
Virkon S	2% solution		3/3	3/3	No Interference
Isopropyl Alcohol	100%		3/3	3/3	No Interference
Nutrients					
General Hydroponics FloraNova Growth	Prepared according to manufacturer instructions	Root tissue submerged in solution for 30 secs and added directly to sample tube	3/3	3/3	No Interference
General Hydroponics FloraNova Bloom	Prepared according to manufacturer instructions		3/3	3/3	No Interference
CloneX	1:1 solution with distilled water		0/3	0/3	Interference
IPM Chemical Treatment					
Bon-Neem	Full Strength	Root tissue submerged in solution for 30 seconds and added directly to sample tube	3/3	3/3	No Interference
Dyna-Gro Neem Oil	Manufacturer instructions		3/3	3/3	No Interference
Mammoth CannaControl	Manufacturer instructions for severe outbreak		3/3	3/3	No Interference
Green Cleaner	Manufacturer instructions for heavy infestation		3/3	3/3	No Interference
Athena IPM	Manufacturer instructions Treatment Strength		3/3	3/3	No Interference
PyGanic	Manufacturer instructions		3/3	3/3	No Interference
Silver Bullet Micro-ionized Sulphur	Manufacturer instructions		3/3	3/3	No Interference
Impello- Tribus Grow	Manufacturer instructions for heavy treatment		3/3	3/3	No Interference

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Most tested substances showed no interference in the TUMIGlow-HlVd assay. However, results indicated that high concentrations of certain compounds were inhibitory or partially inhibitory to the assay. To formulate recommendations for cultivation facilities using these chemicals, we performed follow-up studies and recommendations as described below.

Substance	Concentration	Details	Successful Tests HLVd -	Successful Tests HLVd +	Conclusion
Disinfectants					
Soil	~ 150mg per sample	Added directly to sample tube	0/3	0/3	Interference
	~ 50mg per sample (wet)		3/3	3/3	No Interference
Rock wool	~ 50mg per sample (dry)	Added directly to sample tube	0/3	0/3	Interference
	~ 20mg per sample (dry)		3/3	3/3	No Interference

Recommendations: While very high concentrations of both soil and rock wool were inhibitory, lower amounts of these compounds did not interfere with the test. It is recommended to minimize the amount of soil or rock wool added to the collection tube by gently wiping away material attached to the root prior to testing. Sampling soil-grown plants when the medium is dry is also recommended.

Substance	Concentration	Details	Successful Tests HLVd -	Successful Tests HLVd +	Conclusion
Disinfectants					
Chlorox Bleach	20% solution of 7.5% sodium hypochlorite	Fully submerged scissors used to cut root without rinse	0/3	0/3	Interference
Chlorox Bleach	20% solution of 7.5% sodium hypochlorite	Fully submerged scissors were dabbed lightly with paper towel without rinse	3/3	3/3	No Interference

Recommendations: Bleach is very effective at degrading viroids and other DNA/RNA molecules, meaning bleach added directly to the sample tube will compromise the integrity of the sample. During tissue sampling, it is recommended to briefly dip bleach sterilized scissors in clean water or dab excess bleach from the scissors prior to sampling.

Substance	Concentration	Details	Successful Tests HLVd -	Successful Tests HLVd +	Conclusion
Nutrients					
CloneX	1:1 solution with distilled water	Root tissue submerged in solution for 30 secs and added directly to sample tube	0/3	0/3	Interference
CloneX	1:20 solution with distilled water		3/3	3/3	No Interference
CloneX	1:100 solution with distilled water		3/3	3/3	No Interference

Recommendations: High concentrations of Clone X interfere with the TUMIGlow assay. Due to the nature of how this product is used, only residual amounts of Clone X are expected to remain in tissue taken from a well rooted clone. However, if large amounts of Clone X are present, it is recommended to rinse root tissue in clean water prior to adding to the sample tube.

Technical Accuracy

A technical accuracy test determines the accuracy of an assay compared to an alternative, high sensitivity test. An acceptable technical accuracy shows at least 95% agreement between results obtained from the assay in question and a comparison method.

To determine technical accuracy, 105 independent samples were prepared, 45 HLVd positive samples with variable HLVd levels, 30 HLVd negative samples and 30 failed (no template) samples. Samples were coded and tested by a blinded operator using both TUMIGlow-HLVd on-site assay and by TUMI Genomics HLVd qRT-PCR.

As shown by the table below, **the TUMIGlow-HLVd assay performed with 99.1% accuracy compared to PCR.** The TUMIGlow test detected 100% of HLVd(+) samples down to ~20 viroid copies/µL. Additionally, no false positive or false negative results were obtained.

Sample	Fraction Positive (TG)	Fraction Negative (TG)	Fraction Failed (TG)	Control CT (PCR)	HLVd CT (PCR)	Viroid Load (genomes/ µL)*	Accuracy
High-HLVd+	15/15	0/15	0/15	28.5 +/- 1.2	24.0 +/- 0.5	2649.2	100%
Mid-HLVd+	15/15	0/15	0/15	30.7 +/- 0.9	27.4 +/- 1.3	502.4	100%
Low-HLVd+	15/15	0/15	0/15	31.2 +/- 0.2	31.4 +/- 1.9	27.8	100%
Negative	0/30	29/30	1/30	32.67 +/- 0.8	NaN	NA	96.7%
No Tissue	0/30	0/30	30/30	NaN	NaN	NA	100%

Infield Evaluation

The purpose of an infield analysis is to determine the accuracy of a test when performed by non-scientists in the intended setting (i.e. the test is performed by cultivators in a cannabis growing facility). The infield accuracy of the TUMIGlow-HLVd test was determined by comparing the results of TUMIGlow assay to TaqMan qRT-PCR using samples collected in four independent cultivation facilities. For each plant tested, two tissue samples were collected. One collected sample was used to run the TUMIGlow_HLVd test on-site and the other sample was tested by qRT-PCR at the TUMI Genomics laboratory.

A total of 807 plants produced usable results by both TUMIGlow and qPCR. Among the samples, 117 tested positive for HLVd by PCR. Testing of the paired samples using TUMIGlow found 111 of the 117 positive samples were also positive by TUMIGlow (94.9%). Further analysis of the six disparate results indicated that three showed a viroid load well below the limit of detection of the TUMIGlow-HLVd assay (< 4 viroid particles per uL, CT >33).

Among the 756 samples that tested negative for HLVd by PCR, 751 (99.3%) also tested negative by TUMIGlow.

- TUMIGlow successfully identified 94.9% of all true positives and 97.4% of all positives within the limit of detection of the test.
- For true negative samples, TUMIGlow correctly identified 99.3% of the samples as negative.

	PCR Result		Overall % Agreement
TUMIGlow Test Result	Positive	Negative	
Positive	111	5	98.6%
Negative	6	751	
Total Tested	117	756	
Percent Agreement (95% Confidence Interval)	94.9% (89.0% - 97.9%)	99.3% (98.4% - 99.8%)	

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During the infield study, we noted some portion of the samples did not amplify the internal cannabis control producing a "Failed" result with the TUMIGlow-HLVd test. A series of adjustments were made to correct this issue. A subset of the samples included in this study were run using these adjusted conditions to confirm an acceptable rate of failed samples.

Of the 482 samples that were tested using the adjusted parameters, 28 samples produced a failed result in the TUMIGlow-HLVd test (5.8%). Upon direct rerun of these samples, only 6 produced a failed result (1.2%). Inspection of these samples indicated that they either contained large amounts of coco/rockwool contamination or excessive root tissue. The rate of failed samples tended to decrease as users become comfortable running the test, suggesting initial failed results decrease as the operator becomes comfortable with the procedure.

Flexibility Studies

Flexibility studies determine the limitations of a test so users can understand whether specific deviations from the instructions can be tolerated or will result in decreased accuracy. High flexibility in a test makes it more likely a non-expert user will be able to complete the testing process and obtain an accurate result.

The boundaries of the TUMIGlow-HLVd test were determined by deviating from the recommended procedure for each step in the instructions. The flexibility range shows the extent to which a user can stray from the written instructions and still produce an accurate result. Each study was performed in triplicate with both HLVd negative samples and samples spiked with 3X the limit of detection of the assay. These studies show that the TUMIGlow-HLVd assay has a wide range of flexibility at every step of the testing process, indicating that the test is simple enough to be accurately performed by non-scientists.

Step or Condition	Recommended Procedure	Flexibility Range
Amount of root tissue added to collection tube	Add 3 pieces of root to sample collection tube	1-6 pieces of clean root tissue roughly the diameter of the collection tube
Collected sample stability	Use samples within 3 hours	6 hours at room temperature 24 hours refrigerated
Volume of sample added to the reaction ball	40µL	25µL – 60µL (a 37% decrease up to a 50% increase)
Stability of the reaction with sample added, prior to incubation in the heat block	Put reactions in heat block within 30 minutes after adding sample	0 minutes – 120 minutes
Heat block incubation time	90 minutes	45 minutes – 120 minutes
Continual heat block incubation	Heat reactions continually for 90 minutes	0 – 1 break in incubation where reactions cool to room temperature prior to being reheated for 90 minutes
Result stability of completed reactions	View reactions within 1 hour of heat block shutting off	0-24 hours after heat block shuts off automatically

TUMI Genomics Lead Scientists

Tassa Saldi, PhD: Dr. Saldi received her undergraduate and graduate degrees in molecular biology from the University of Colorado in Boulder and completed her post-doctoral studies at the Health Sciences Center, University of Colorado, Denver. Her graduate work explored the molecular mechanism underpinning Amyotrophic Lateral Sclerosis (ALS) and the role of double-stranded RNA accumulation and heterochromatin in pathogenesis.

Continuing her work on structured RNA during post-doctoral work, Dr. Saldi investigated the role of genome-wide nascent RNA secondary structure in co-transcriptional splicing, A-to-I RNA editing and transcription termination. Her work was supported by fellowships from the American Cancer Society and the RNA Biosciences Intuitive (RBI). Following her postdoc, Dr. Saldi directed the COVID-19 surveillance lab at CU, Boulder where she supervised a team of 8 scientists and designed and validated multiple PCR assays to detect SARS-CoV-2 in human saliva. She is a lead scientist and CSO of TUMI Genomics.

Her publications can be found here: https://pubmed.ncbi.nlm.nih.gov/term=Tassa+Saldi&sort=date

Alfonso Garrido-Lecca, PhD: Dr. Garrido-Lecca received an undergraduate degree in biology with a minor in chemistry from Texas A&M University. He pursued his PhD at the University of Colorado, Boulder in molecular biology. His graduate work focused on using the unique genetic organization of C. elegans to understand how genes are expressed and RNA transcripts processed. His postdoctoral work focused on the regulation of microRNAs in leukemia and was supported by a fellowship from the Linda Crnic Institute for Down Syndrome and the National Institute of Health T32 training grant. Dr. GarridoLecca is a lead scientist at TUMI Genomics and head of Research and Development.

His publications can be found here:
https://pubmed.ncbi.nlm.nih.gov/term=alfonso+garridolecca&sort=date

Aisha Jama, *MS:* Aisha Jama is an experienced scientist with expertise in microbiology, molecular biology and analytical chemistry. She holds a Master of Science in Soil and Crop Science from Colorado State University where she researched organic fertilizer methods and authored peer review research. Aisha brings years of experience conducting agriculture pathogen testing, adherance to GLP and GMP standards and creation and management of rigorous laboratory SOPs. As laboratory manager, Aisha ensures the highest quality standards are enforced at TUMI Genomics laboratory.

Her publications can be found here: https://www.mdpi.com/2071-1050/15/7/6045

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