Validation Summary 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://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

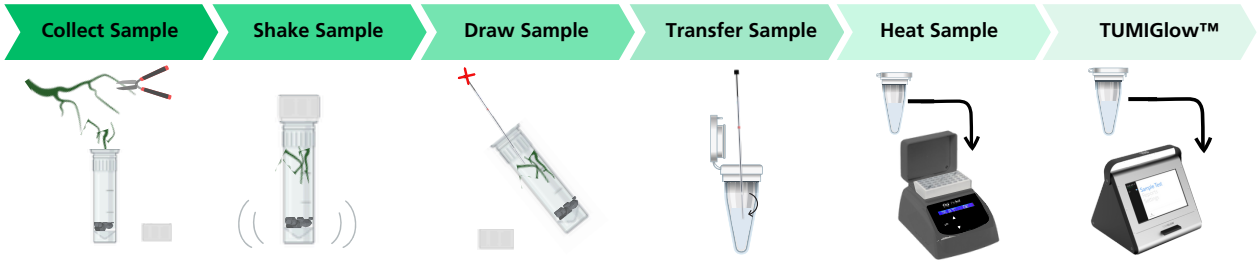
TUMI Genomics' on-site hop latent viroid (HLVd) assay, TUMIGlow, detects the presence of HLVd infection in cannabis/hemp plant tissue. The TUMIGlow-HLVd test is based on Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) technology. However, several key changes to standard RT-LAMP 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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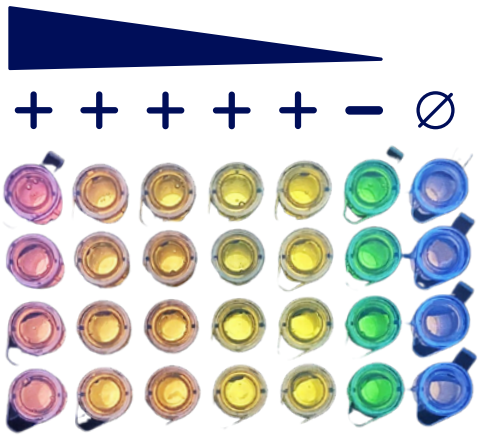
Result Analysis and Tracking: TUMIGlow-HLVd tests are analyzed by the Glow Device. The Glow Device software can determine the results of 48 TUMIGlow-HLVd tests in less than a minute, allowing hundreds of samples to be tested in a day. Results are displayed in a spreadsheet like format and select results 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



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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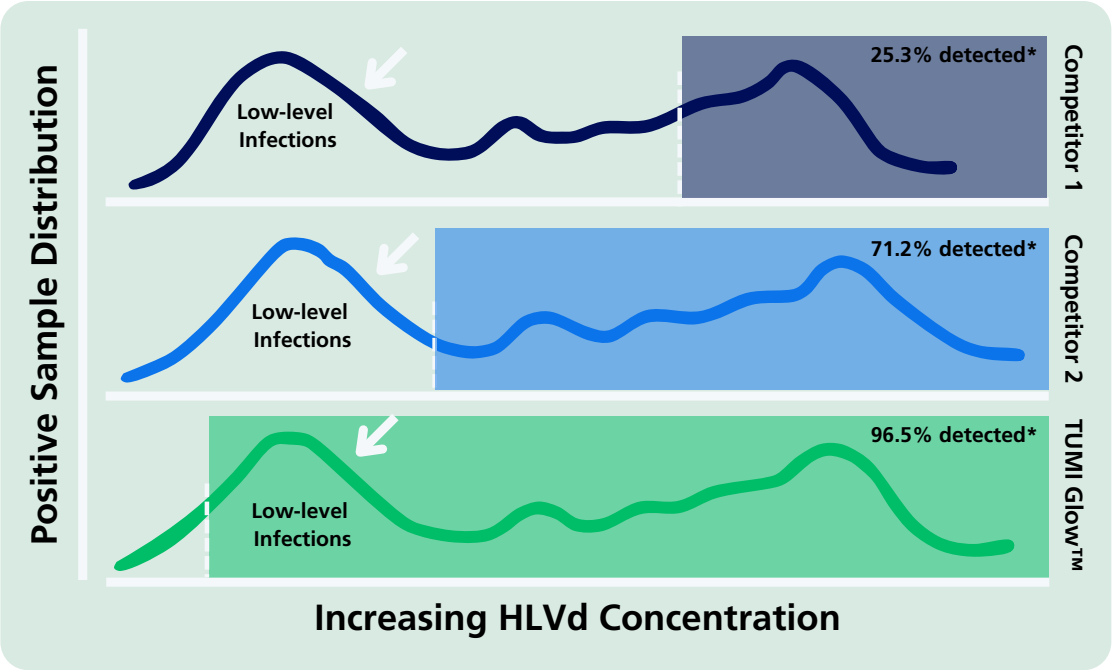
Validation Summary

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 of 135 positive samples from customers indicates 99.3% detection of samples 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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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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