



Agdia Releases Rapid Isothermal Molecular Assay for Detection of HLVd (Hop Latent Viroid)

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Agdia, Inc. (Elkhart, IN) has commercialized a rapid and user-friendly, RNA-based assay, on their AmplifyRP® XRT platform, for the [detection and identification of Hop latent viroid \(HLVd\)](#).

Hop latent viroid (HLVd, *Cocadviroid*) is widespread, infecting cultivated hops, *Humulus lupulus*, worldwide. This pathogen was discovered during surveys for *Hop stunt viroid* (HSVd, *Hostuviroid*) in Spain and Germany during the late 1980's. Initially, HLVd was referred to as "hop viroid-like RNA fast, or HV-f." Thereafter, it was characterized and named *Hop latent viroid*. In addition to cultivated hops, HLVd is known to infect Japanese hops, *H. japonicus*, and *Cannabis sativa*.

Early research with HLVd suggested minor deleterious effects on most commercial varieties of hops; typical infected plants were asymptomatic, hence the latent designation. Nevertheless, varieties such as Omega did express chlorosis, decreased growth rates and biomass, and decreased cone size and number, due to HLVd infection. Current research suggests that HLVd infections can significantly impact the production of secondary metabolites and alpha acids used in beer production, on several hop varieties. Therefore, the infections caused by HLVd in many hop varieties are not latent, as once believed.



Figure 1: Agdia's AmpliFire® portable isothermal fluorometer

Hop latent viroid is known to cause a combination of symptoms on *Cannabis sativa*, including stunting, chlorosis, leaf deformation, brittle stems, reduced flower mass and trichome number, and alteration of typical plant morphology. This symptomology is commonly referred to collectively as "dudding." Research on the *Cannabis* pathosystem is relatively new, and as *Cannabis* production has scaled up, plant disease symptoms hitherto unnoticed have become limiting factors. Such is the case for HLVd, and broad characterization of management and epidemiology of this pathogen on this crop has yet to be accomplished.

The widespread distribution of HLVd is due to the movement of infected, but asymptomatic propagative materials in hops. The movement of infected propagative materials in *Cannabis* has surely contributed to the distribution of HLVd in this crop; however, the extent of distribution between and within *Cannabis* inventories is unknown. *Hop latent viroid* is spread efficiently via mechanical transmission on tools and equipment. Therefore, movement within a hop yard or a *Cannabis* facility is facilitated by activities such as pruning and harvesting. Moreover, seed and pollen transmission have been reported, but are not considered important to the epidemiology of either pathosystem. Finally, HLVd transmission via arthropod vectors or encapsidation by co-infecting viruses are not well-characterized; more research is required for comprehensive understanding of these vectoring paradigms.



As with viruses, no curative therapies exist for viroid infections in mature plants. Therefore, exclusion is the most important viroid management strategy, and diagnostic testing is the cornerstone of an effective management program. The burgeoning industries of hop and *Cannabis* production have elucidated the necessity of a reliable and robust diagnostic for detection of HLVd in plant inventories. Moreover, regulations regarding the interstate movement of *Cannabis* have constrained many growers to identify infections at farm level, making the field-based usability of a diagnostic product indispensable.

Agdia's new [AmplifyRP® XRT assay for detection of Hop latent viroid](#) is based on recombinase polymerase amplification (RPA). This technology promotes the rapid amplification and detection of nucleic acid targets, DNA or RNA, while maintaining a single operating temperature of 39° – 42°C. The AmplifyRP® XRT products achieve target sensitivity and specificity comparable to PCR, while having clear advantages over the lab-based technology. AmplifyRP® XRT products do not require a nucleic acid purification step; crude sample extracts are prepared using a simple extraction buffer and tested directly. This makes the testing process simple and saves the end user valuable time. Moreover, this facilitates the implementation of this technology at remote locations with limited resources. When paired with Agdia's AmpliFire® isothermal fluorometer, the XRT system is a rapid, user-friendly tool that can be implemented in the field or the lab by personnel with limited experience in molecular diagnostics.

Agdia states their assay is specific to HLVd and was screened against a diverse collection of confirmed isolates infecting hops and *Cannabis*, showing 100% accuracy in detection of true positives. Moreover, the product exhibited no cross-reactivity with an extensive panel of common viroid and viral pathogens, including *Apple mosaic virus*, *Arabidopsis mosaic virus*, *Citrus bark cracking viroid*, *Coconut cadang-cadang viroid*, *Coconut tinangaja viroid*, *Columnnea latent viroid*, *Hop latent virus*, *Hop mosaic virus*, *Hop stunt viroid*, *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid*. Sensitivity for this assay is comparable to that observed with the published RT-qPCR assay and greater than the published conventional RT-PCR assay to which it was compared. This product was developed to test leaf and petiole tissue.

Agdia now offers AmplifyRP® XRT products for detection of the two most widely distributed viroid pathogens present in hops, [HSVd](#) and [HLVd](#). The introduction of this product brings Agdia's catalog to 21 assays on the AmplifyRP® platform. High levels of market demand for field-deployable, plant pathogen detection products have driven this output, and Agdia maintains they will continue to expand their product offerings. For more information on Agdia's complete line of AmplifyRP® assays, please visit Agdia's website www.agdia.com.

About Agdia

A leading provider of diagnostic solutions for agriculture, Agdia, Inc. has been serving plant breeders, propagators, growers, universities, regulatory organizations and private testing laboratories since 1981. The company offers a comprehensive portfolio of validated, easy-to-use diagnostics for identifying plant pathogens, plant hormones, and transgenic traits. Moreover, Agdia operates an ISO accredited, in-house, testing services laboratory. Agdia's quality management system is ISO 9001:2015 certified and their Testing Services Laboratory is ISO 17025:2017 accredited. Visit the company's website at



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About AmplifyRP®

AmplifyRP® XRT and AmplifyRP® Acceler8® test kits employ recombinase polymerase amplification (RPA) technology licensed by Agdia, Inc (licensee) from TwistDx Limited, U.K (licensor). Use of the RPA process and probe technologies are protected by US patents 7,270,981 B2, 7,399,590 B2, 7,435,561 B2, 7,485,428 B2 and foreign equivalents in addition to pending patents.

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