



Grapevine Virus Testing Methodologies

By Judit Monis, Ph. D

Above Photo: Eurofins STA laboratory technician preparing a plate for a RT-PCR analysis run

To keep our laboratory up to the highest standards, we stay current with scientific literature and technologies. Our friendly local and international collaborations allow us to access new information and quickly add to our testing menu. A couple of months ago, a group at UC Davis presented comparative data on testing methods used in their laboratory. The group claims that Taqman is the most sensitive technique available for detecting grapevine viruses. Following the presentation, we received a high number of calls from concerned clients wanting to know if we are using the technique and how does it compare to our present testing practice. In short, the technique is not new - it has been around for over eighteen years. Second, Taqman has its own limitations as a detection method and would need to be implemented as a complementary test. Lastly, this additional test could add a significant cost to our present

pricing structure. In light of our clientele interest, this quarterly newsletter issue is dedicated to explaining the techniques we use and where Taqman fits in.

Two methods are standard for the detection of viruses in grapevines: ELISA and RT-PCR. ELISA stands for "enzyme-linked immunosorbent assay, and works by using antibodies to react directly with the virus of interest on a test plate. After the antibodies bind, an enzyme is added that changes the reacted region's color. By detecting a color change (not as simple as it sounds -an ELISA reader must be used to reliably determine the results), the presence of virus is confirmed. Because ELISA uses antibodies, it is very broad in detecting a diverse population of virus. The drawback is that detection is limited to the amount of virus present in the test sample and sometimes the titer (or concentration) may be too weak to detect.

RT-PCR is short for "reverse transcription-polymerase chain reaction. The RT-PCR technique allows the amplification (i.e., makes multiple copies) of viral RNA extracted from the vines. The amplification is repeated many times with each copy making more copies of itself. After the completion of the assay, over a billion copies of the viral RNA are produced. It is a workhorse in situations when the virus is found in low concentration and is difficult to detect using ELISA. Relative to grapevines, clearly the art of extracting virus from the test sample is critical to success, which explains why some labs are more successful with this technique than others. Because PCR amplifies the targeted viral genome, it is susceptible to contamination problems. A clean lab is critical to running PCR to avoid cross-contamination

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problems. Lastly, while RT-PCR is extremely selective in terms of exact sequence of RNA, it may miss slight changes (mutations) in virus populations (variant strains). Diversity in the genome information of viruses is not rare, but rather the rule in nature since each time a virus replicates (the reproduction cycle in viruses) mistakes in the copy cycle occur (just as we have small variations in our own DNA compared to our parents' DNA!) creating genomic variants. For this reason, ELISA remains a critical complementary technique for detecting viruses that can't be detected by RT-PCR.

Taqman is a specific type of PCR. It is named after the bacterium *Thermus aquaticus* (Taq) from which the Taq polymerase was isolated from and its "PacMan" (videogame) nature. Its key advantages over RT-PCR are that it can be used to relatively quantify the titer of virus instead of a yes/no presence of virus and does not require laborious post-PCR detection procedures (reduces the potential of lab contamination). Its disadvantages compared to RT-PCR is its inability to monitor the amplified fragment size (a way of

verifying the right POSITIVE result), limitation in the availability of reliable probes, and the significant expense both to develop and run. Note that increased sensitivity is not included because it is not significantly different.

In sum, ELISA works by using antibodies to attach to a virus while PCR-based methods, RT-PCR/Taqman use RNA molecular probes with exact molecular matches. ELISA is less sensitive but broader in its use while the PCR-based methods are very sensitive to low virus concentrations, but incapable of detecting viral variants. RT-PCR is effective for "yes/no" presence of virus while Taqman is better at relatively quantifying the amount of virus present. Lastly, Taqman does not seem to add a detection advantage compared to RT-PCR, but is significantly more expensive to implement. In spite of this, our lab plans to do a deep in-house comparative study between these two molecular techniques. If our results suggest that the method is superior, we would include Taqman in our present arsenal of tests offered to the vineyard industry (see <http://www.stalabs.com/products-services-grapevine-testing.html>).

com/products-services-grapevine-testing.html).

Please call us if you like to discuss testing needs or have questions about the testing methods used for the detection of grapevine viruses.



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STA Laboratories

Colorado Laboratory

1821 Vista View Drive
Longmont, Colorado 80504
(303) 651-6417

Gilroy Laboratory

7240 Holsclaw Rd.
Gilroy, CA 95020
(408) 846-9964

www.eurofinsus.com/stalabs
stalabs@eurofinsus.com