# Development of a quantitative PCR assay for the detection of *Grapevine Red Blotch Virus*

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## Introduction

*Grapevine red blotch virus* (GRBV) is an emerging grapevine virus causing a non-curable and spreading disease. Typical symptoms caused by GRBV infection on red cultivars is reddening of leaf blade. Fruit quality on diseased vines is also impacted compared to healthy controls (Reynard et al, 2018). Grapevine red blotch virus is widespread in North America (Krenz et al., 2014). Due to its wide occurrence, transmissibility and impacts on grape quality, this virus has the potential to cause serious economic losses.

Photos showing typical red blotch symptoms on a red cultivar Gamay (top) and 2 different white cultivars (bottom left is Sauvignon blanc and bottom right is Chardonnay).

*Photos by Jean-Sébastien Reynard at Agroscope, and Eurofins Biodiagnostics USA.*

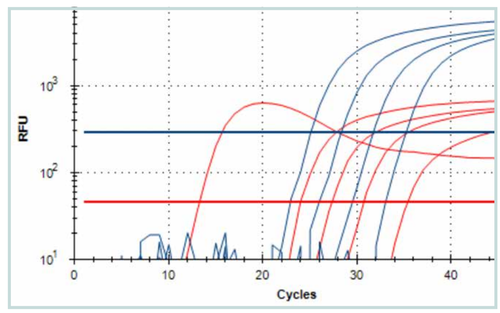
## Material and Methods

All 27 complete GRBV genomes available at NCBI (stand May 2016) were aligned using Geneious R10. Several quantitative PCR assays (TaqMan) were designed in conserved regions. A host gene from Vitis vinifera was used in duplex as internal PCR control. All combinations were tested using healthy and infected grapevine samples as well as positive and negative controls available in the grapevine virus collection at Agroscope. DNAs from these samples were extracted using a rapid CTAB method. The best primers/probe setting based on sensitivity and specificity of detection was chosen to be deeper validated with the help of key laboratories in USA. Sampling will also be studied through the year to determine the best period and material (e.g. bark scrapings, young vs mature leaf) to be used for detection (ongoing work at Agroscope).

## Results

**A.**

| | Cq vFAM (GRBV, blue) | Cq Cy5 (Vitis gene, red) |
|---|---|---|
| **GRBV+ $10^{-3}$** | 23.7 | 24.5 |
| **GRBV+ $10^{-4}$** | 27.0 | 27.6 |
| **GRBV+ $10^{-5}$** | 30.3 | 31.0 |
| **GRBV+ $10^{-6}$** | 33.9 | 35.5 |
| **Healthy** | n/a | 13.1 |
| **NTC** | n/a | n/a |




**B.**

| | Cq vFAM (GRBV) | Cq Cy5 (Vitis gene) |
|---|---|---|
| **Clade 1 #1** | 17.2 | 14.8 |
| **Clade 1 #2** | 23.7 | 25.3 |
| **Clade 1 #3** | 16.6 | 15.0 |
| **Clade 2 #1** | 15.6 | 13.1 |
| **Clade 2 #2** | 31.8 | 29.1 |
| **Clade 2 #3** | 24.0 | 20.7 |
| **Healthy** | n/a | 15.3 |
| **NTC** | n/a | n/a |

**A.** Dilution serie of a GRBV-positive sample showing amplification of GRBV (FAM, blue curves) and a host gene from *Vitis vinifera* (Cy5, red curves). DNA extracted from a non-infected grapevine (healthy) shows amplification only for the host gene. NTC= No Template Control.

**B.** The assay was tested on samples from infected vineyard plots in California (clade 1 #2=Merlot, clade 1 #3=Cabernet Sauvignon, clade 2 #2=Merlot and clade 2 #3=Cabernet franc), while clade 1 #1 (Syrah) and clade 2 #1 (Chardonnay) samples come from vines infected following agroinoculation with infectious clones of GRBV. The healthy vine sample corresponds to Syrah vines grown in vitro.

## Conclusion

- The assay allows the detection of GRBV in infected grapevine samples with a good sensitivity. The virus is still detectable in a DNA extract diluted $10^6$x (starting material: 0.5 g of infected tissue).
- Detection of representative variants from the two distinct phylogenetic groups of GRBV.

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