LE GOUVERNEMENT DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture, de la Viticulture et de la Protection des consommateurs
Administration des services techniques de l'agriculture

Multiplex qPCR virus detection on dormant seed potato tubers using a rapid RNA extraction method

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Introduction

Luxemburg's seed potato production comprises about 450 ha per year and primarily covers a high class production. Until 2014, the Luxemburgish virus testing on seed potatoes had been performed with ELISA on tubers, by breaking dormancy with Rindite. An alternative multiplex real-time RT-PCR testing technique for virus detection was assessed. The test detects the most common potato-infecting viruses occurring in Luxemburg, namely PVY and PLRV, furthermore rarely occurring viruses such as PVS, PVX and PVA are also tested.
In cooperation with the SASA institute[1], the experimental conditions have been modified in order to increase analytical throughput. By combining the rapid RNA extraction method from BIOREBA[2] with multiplexing, reliable and time-efficient results have been achieved.

Material and methods

Sampling & RNA extraction: Sampling of dormant tubers with various bulk sizes; homogenization of tuber samples in extraction bags by using Homex 6 (BIOREBA); RNA extraction with rapid extraction buffers (BIOREBA) according to manufacturer's protocol
Real-time RT-PCR: LightCycler 96 (Roche)

Data analysis: Statistical analysis with Seedcalc8 (ISTA online[3])

Primers and probes selection (previously prescribed): **PVY** and **PLRV**: Boonham et al. (2009)[4], **PVS**: Mortimer-Jones et al. (2009)[5], **PVX**: Agindotan et al. (2007)[6]; Mortimer-Jones et al. (2009)[5], **PVA**: Lacomme (2015)[7]; performed in duplex for PVY-PLRV and triplex for PVS-PVX-PVA

Schema of rapid extraction

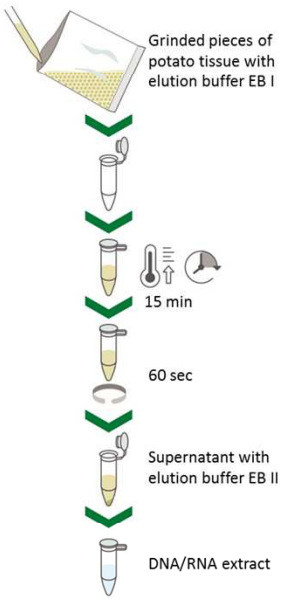


Figure 1

Figure 2: Grinding tissue

Figure 3: Transfer of homogenate

Figure 4: Executing RT-PCR

Reproducibility: Testing of different tissues, 2 operators , 2 days

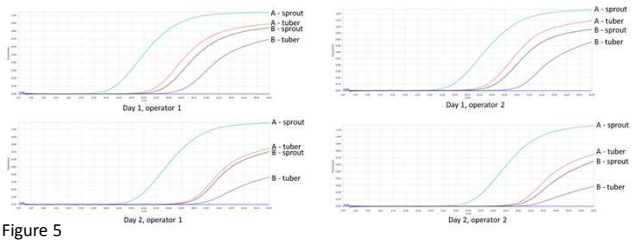
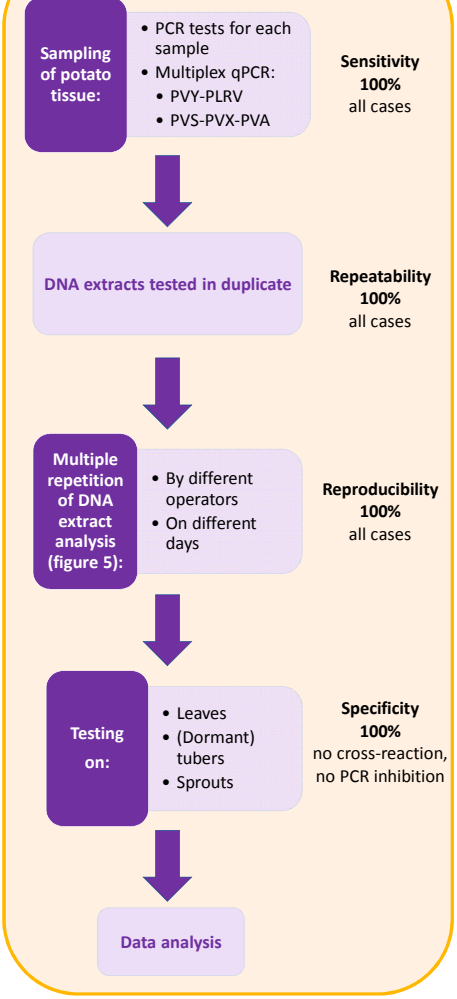


Figure 5



Results and discussion

The sampling method has been evaluated with respect to EPPO[8] performance criteria including sensitivity and repeatability (i.e. sampling of tuber cores at the stolon- and rose-end for each tuber, pool size of 10-20 tubers).

A rapid RNA extraction method has been assessed that allows nucleic acid extraction in about 30 minutes (figure 1). This method is suitable for (semi-)automated handling (Figures 2-4), increasing its throughput.

RNA extracts can be stored up to several days at -20°C without affecting significantly the performance of the test.

The combinations of primers and probes were evaluated individually and in multiplex. The duplex PVY-PLRV and triplex PVS-PVX-PVA combinations show similar results as those performed individually (figure 6) and therefore were selected for future assays.

The method can be applied to different types of potato tissues, such as leaves, (dormant) tubers or sprouts.

Sensitivity: Individual testing vs. multiplex

Individually performed qPCR (PVX) in comparison with triplex combination (PVX-PVA-PVS) and dilution 30-fold and 60-fold with healthy homogenate

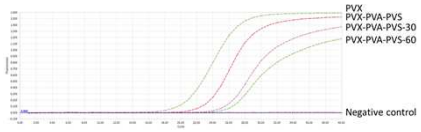


Figure 6

Conclusion

- The use of the multiplex virus detection method with RT-PCR, in combination with the developed sampling method and the simplified RNA extraction, enables sensible and repeatable results.
- This method can be applied directly on dormant tubers, reducing the turnover in comparison to the growing-on ELISA test (about 6 weeks), which is still used for post-harvest testing.
- The multiplex qPCR allows a quick assessment of virus incidence and is used for routine testing in Luxemburg since 2015.

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