A revised report of Euphresco 2019-A-327 "Validation of diagnostic tests for the detection and identification of Tomato brown rugose fruit virus in tomato and pepper seeds" was published in April 2022. This revised report includes additional data to improve transparency and detailing of the conclusions of the test performance study.

Detailed changes are listed below:

Page 9

Previous text:

"In contrast, end-point RT-PCR and isothermal amplification tests appeared unsuitable for the reliable detection of ToBRFV in tomato and pepper seeds, because they were not sensitive enough."

New text:

"End-point RT-PCR and isothermal amplification tests assessed in this study showed a lower diagnostic sensitivity than the real-time RT-PCR tests, resulting in a higher number of false negative results. However, the isothermal amplification tests may require further evaluation by a greater number of participating labs."

Page 11

Previous text:

"In contrast, DAS-ELISA, end-point RT-PCR and isothermal amplification tests as assessed in this study, appeared unsuitable for reliable detection of ToBRFV in tomato and pepper seeds, because they were not sensitive enough. However, for the isothermal amplification tests this conclusion was based on a limited number of data sets."

New text:

"DAS-ELISA, end-point RT-PCR and isothermal amplification tests assessed in this study showed a lower diagnostic sensitivity than the real-time RT-PCR tests, resulting in a higher number of false negative results. However, the isothermal amplification tests may require further evaluation by a greater number of participating labs."

<u>Page 14</u>

Additional text:

"Data of the test performance study provided by participants (anonymized) are available at: <u>https://zenodo.org/record/6247412#.YhZ0a-jMKUk"</u>

Page 34

Previous text:

"A few participants used different kits/enzymes than prescribed for the TPS for some of the tests (ISHI-Veg: 2 + 1 unreported, M&W: 1 + 1 unreported, Alkowni: 2). The results of only one test produced with a different kit were outliers."

New text:

"A few participants used different kits/enzymes than prescribed for the TPS for some of the tests (ISHI-Veg: 4 + 1 unreported, M&W: 1 + 1 unreported, Alkowni: 2). The results of only two tests produced with a different kit were outliers."

Page 35-36

Figure 4 and Table 12 were slightly adjusted.

Page 36

Additional text:

"Appendix 10 shows that this is also the case when datasets with >10% undetermined/missing results and outlier datasets are included."

Page 41

Previous text:

"In contrast, DAS-ELISA, end-point RT-PCR and isothermal amplification tests as assessed in this study, appeared unsuitable for reliable detection of ToBRFV in tomato and pepper seeds, because they were not sensitive enough."

New text:

"DAS-ELISA, end-point RT-PCR and isothermal amplification tests assessed in this study showed a lower diagnostic sensitivity than the real-time RT-PCR tests, resulting in a higher number of false negative results. However, the isothermal amplification tests may require further evaluation by a greater number of participating labs."

Page 79:

Appendix 10 was added.