**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**
**ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES**
**Summary sheet of validation data for a diagnostic test**

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

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| **Laboratory contact details** | Netherlands Institute for Vectors, Invasive plants and Plant health<br>P.O. Box 9102, 6700 HC Wageningen, Netherlands |
| **Short description of the test** | This test can be used for the untargeted detection and identification of molecularly characterized ssRNA(+), ssRNA(-), dsRNA, cssRNA, dsDNA(-RT), ssDNA viruses and viroids in symptomatic plant samples. |
| **Date, reference of the validation report** | 2020-07-13 - 2020.molbio.004 v1, 2021.molbio.009 v3 |
| **Link to other validation data** | - 2020.molbio.012 This validation data is for generic detection and identification of phytoplasmas. Phytoplasmas can be detected using conventional nested PCR. The conventional (nested) PCR product is purified and finally sequenced using HTS. For identification see validation sheet 571. |
| **Validation process according to EPPO Standard PM7/98?** | yes |
| **Is the lab accredited for this test?** | yes |
| **Was the validated data generated in the framework of a project?** | no |
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| **Description of the test** | |
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| **Organism(s)** | Cocadviroid cadangi (CCCVD0)<br>Cowpea mild mottle virus / Carlavirus vignae (CPMMV0)<br>Lettuce infectious yellows virus / Crinivirus lactucaflavi (LIYV00)<br>Peach mosaic virus / Trichovirus persicae (PCMV00)<br>Dichorhavirus orchidaceae (OFV000)<br>Peach rosette mosaic virus / Nepovirus persicae (PRMV00)<br>Potato black ringspot virus / Nepovirus solani (PBRSV0)<br>Pepino mosaic virus / Potexvirus pepini (PEPMV0)<br>Potato yellowing virus (PYV000)<br>Ilarvirus SNSV (SNSV00)<br>Physostegia chlorotic mottle virus / Alphanucleorhabdovirus physostegiae (PHCMOV)<br>Potato virus X / Potexvirus ecspotati (PVX000)<br>Potato virus Y / Potyvirus yituberosi (PVY000)<br>Sri Lankan cassava mosaic virus / Begomovirus stanleyi (SLCMV0) |

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| | Potato leafroll virus / Polerovirus PLRV (PLRV00)<br>Potato virus A / Potyvirus atuberosi (PVA000)<br>Emaravirus rosae (RRV000)<br>Crinivirus ipomeae (SPCSV0)<br>Carlavirus chisolani (PVH000)<br>Potato virus T / Tepovirus tafsolani (PVT000)<br>Tobacco ringspot virus / Nepovirus nicotianae (TRSV00)<br>Potato virus M / Carlavirus misolani (PVM000)<br>Potato virus S / Carlavirus sigmasolani (PVS000)<br>Squash vein yellowing virus / Ipomovirus cucurbitavenaflavi (SQVYVX)<br>Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV)<br>Tomato leaf curl New Delhi virus / Begomovirus solanumdelhiense (TOLCND)<br>Tomato yellow leaf curl Thailand virus / Begomovirus solanumflavusthailandense (TYLCTH)<br>Viruses and viroids (1VIRUK)<br>Potato yellow vein virus / Crinivirus flavisolani (PYVV00)<br>Tomato golden mottle virus / Begomovirus solanumaureivariati (TOGMOV)<br>Tomato mottle mosaic virus / Tobamovirus maculatessellati (TOMMV0)<br>Tomato mild mottle virus / Ipomovirus lycopersici (TOMMOV)<br>Tomato ringspot virus / Nepovirus lycopersici (TORSV0)<br>African cassava mosaic virus / Begomovirus manihotis (ACMV00)<br>Andean potato mild mosaic virus / Tymovirus mosandigenum (APMMV0)<br>Alfamovirus AMV (AMV000)<br>Andean potato mottle virus / Comovirus andesense (APMOV0)<br>Arabis mosaic virus / Nepovirus arabis (ARMV00)<br>Bean golden yellow mosaic virus / Begomovirus birdi (BGYMV0)<br>Cucumber green mottle mosaic virus / Tobamovirus viridimaculae (CGMMV0)<br>Cucurbit aphid-borne yellows virus / Polerovirus CABYV (CABYV0)<br>Grablovirus vitis (GRBAV0)<br>Andean potato latent virus / Tymovirus latandigenum (APLV00)<br>Arracacha virus B / Cheravirus arracaciae (AVB000)<br>Apple fruit crinkle viroid (AFCVD0)<br>American plum line pattern virus / Ilarvirus APLPV (APLPV0)<br>Beet curly top virus / Curtovirus betae (BCTV00)<br>Beet necrotic yellow vein virus / Benyvirus necrobetae (BNYVV0)<br>Blueberry leaf mottle virus / Nepovirus myrtilli (BLMOV0)<br>Chayote yellow mosaic virus / Begomovirus chayotis (CHAYMV)<br>Cherry rasp leaf virus / Cheravirus avii (CRLV00)<br>Robigovirus robigomaculae (CRMAV0)<br>Robigovirus tortifoliae (CTLAV0) |

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| | Chilli leaf curl virus / Begomovirus chillicapsici (CHILCU)<br>Chrysanthemum stem necrosis virus / Orthotospovirus chrysanthinecrocaulis (CSNV00)<br>Citrus tristeza virus / Closterovirus tristezae (CTV000)<br>Cotton leaf curl Gezira virus / Begomovirus gossypigeziraense (CLCUGV)<br>Cucumber mosaic virus / Cucumovirus CMV (CMV000)<br>Honeysuckle yellow vein virus / Begomovirus macrotylomae (HYVV00)<br>Papaya leaf curl Guandong virus / Begomovirus caricaguandongense (PALCGV)<br>Pepper huasteco yellow vein virus / Begomovirus capsicumhuastecoense (PHYVV0)<br>Potato aucuba mosaic virus / Potexvirus marmoraucuba (PAMV00)<br>Potato spindle tuber viroid / Pospiviroid fusituberis (PSTVD0)<br>Nepovirus betasolani (PVB000)<br>Potato virus P / Carlavirus pisolani (PVP000)<br>potato yellow dwarf virus / Alphanucleorhabdovirus tuberosum (PYDV00)<br>Satsuma dwarf virus / Sadwavirus citri (SDV000)<br>Strawberry latent ringspot virus / Stralarivirus fragariae (SLRSV0)<br>Ipomovirus lenisbatatae (SPMMV0)<br>Tomato chocolàte virus (TOCHV0)<br>Tomato leaf deformation virus / Begomovirus solanumdepravationis (TOLDEV)<br>Tomato marchitez virus / Torradovirus marchitezum (TOANV0)<br>Watermelon chlorotic stunt virus / Begomovirus citrulli (WMCSV0)<br>Begomovirus cocciniae (CMTNV0)<br>Cherry rosette virus (CRV000)<br>Potato virus V / Potyvirus vetuberosi (PVV000)<br>Watermelon silver mottle virus / Orthotospovirus citrullomaculosi (WMSMOV) |
| **Detection / identification** | detection and identification |
| **Matrix(ces) tested** | Flowers, Fruits, Leaves |
| **Plant species tested** | Buddleja davidii, Capsicum, Chenopodium quinoa, Citrus, Cucumis sativus, Fragaria, Malva, Nicotiana benthamiana, Nicotiana occidentalis, Rosa, Rubus idaeus, Salvia, Sambucus nigra, Solanum lycopersicum, Vicia faba |
| **Method(s)** | Molecular HTS |
| **Method: Molecular HTS** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | no |
| **New test being considered for inclusion in the** | yes |

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| **next version of the EPPO diagnostic protocol?** | |
| **As or adapted from an IPPC diagnostic protocol** | no |
| **Reference of the test** | Roenhorst et al. (in preparation) |
| **Is the test modified compared to the reference test** | no |
| ***Other information*** | |
| **Other details on the test** | Included as VirDisc in EPPO PM7/151 - Appendix 1: Example of high throughput sequencing (HTS) tests for the detection and identification of viruses or viroids |
| **Performance Criteria :** | |
| **Organism 1.:** | **Cocadviroid cadangi(CCCVD0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.3x10^1. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 2.:** | **Carlavirus vignae(CPMMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.9x10^2 - 1.8x10^4. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 8 |
| **Specificity value** | 100 |
| **Organism 3.:** | **Crinivirus lactucaflavi(LIYV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.8x10^1. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 4.:** | **Trichovirus persicae(PCMV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 3.8x10^1 - 2.6x10^3. Based on 3 isolates. |
| **Analytical specificity - inclusivity** | |

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| **Number of strains/populations of target organisms tested** | 7 |
| **Specificity value** | 100 |
| **Organism 5.:** | **Dichorhavirus orchidaceae(OFV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.1x10^3 - 2.3x10^3. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 6.:** | **Nepovirus persicae(PRMV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 9.0x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 7.:** | **Nepovirus solani(PBRSV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.9x10^3 - 1.1x10^4. Based on 4 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 4 |
| **Specificity value** | 100 |
| **Organism 8.:** | **Potexvirus pepini(PEPMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.5x10^3 - 6.8x10^3. Based on 3 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | >19 (see annex) |
| **Specificity value** | 100 |
| **Organism 9.:** | **Potato yellowing virus(PYV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.3x10^3 - 5.1x10^3. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |

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| **Number of strains/populations of target organisms tested** | 7 |
| **Specificity value** | 100 |
| **Organism 10.:** | **Ilarvirus SNSV(SNSV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.1x10^3 - 4.2x10^3. Based on 4 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 4 |
| **Specificity value** | 100 |
| **Organism 11.:** | **Alphanucleorhabdovirus physostegiae(PHCMOV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.5x10^2 - 3.3x10^4. Based on 4 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 16 |
| **Specificity value** | 100 |
| **Organism 12.:** | **Potexvirus ecspotati(PVX000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 5.1x10^2. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 13.:** | **Potyvirus yituberosi(PVY000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.4x10^2 - 8.9x10^3. Based on 6 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 43 |
| **Specificity value** | 100 |
| **Organism 14.:** | **Begomovirus stanleyi(SLCMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 8.1x10^1. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |

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| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 15.:** | **Polerovirus PLRV(PLRV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 0.58 - 9.9x10^1. Based on 3 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 13 |
| **Specificity value** | 100 |
| **Organism 16.:** | **Potyvirus atuberosi(PVA000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.0x10^2 - 1.7x10^3. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 7 |
| **Specificity value** | 100 |
| **Organism 17.:** | **Emaravirus rosae(RRV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 3.5x10^1 - 6.7x10^2. Based on 4 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 4 |
| **Specificity value** | 100 |
| **Organism 18.:** | **Crinivirus ipomeae(SPCSV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 8.0x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 15 |
| **Specificity value** | 100 |
| **Organism 19.:** | **Carlavirus chisolani(PVH000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.1x10^4. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |

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| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 20.:** | **Tepovirus tafsolani(PVT000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 7.0x10^1 - 5.1x10^2. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 3 |
| **Specificity value** | 100 |
| **Organism 21.:** | **Nepovirus nicotianae(TRSV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.1x10^2 - 9.0x10^3. Based on 7 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 50 |
| **Specificity value** | 100 |
| **Organism 22.:** | **Carlavirus misolani(PVM000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.6x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 5 |
| **Specificity value** | 100 |
| **Organism 23.:** | **Carlavirus sigmasolani(PVS000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.7x10^3 - 1.5x10^4. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 8 |
| **Specificity value** | 100 |
| **Organism 24.:** | **Ipomovirus cucurbitavenaflavi(SQVYVX)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.8x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |

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| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 25.:** | **Tobamovirus fructirugosum(TOBRFV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 9.1x10^3 - 1.0x10^5. Based on 3 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 299 |
| **Specificity value** | 100 |
| **Organism 26.:** | **Begomovirus solanumdelhiense(TOLCND)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 8.3x10^2 - 5.0x10^3. Based on 3 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 5 |
| **Specificity value** | 100 |
| **Organism 27.:** | **Begomovirus solanumflavusthailandense(TYLCTH)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 4.0x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 28.:** | **Viruses and viroids(1VIRUK)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | To determine the analytical sensitivity, a serial 10 times dilution (10^2 till 10^7) of infected S. lycopersicum homogenate in healthy S. lycopersicum homogenate was made in triplicate. HTS test results show that there is a correlation between the dilution and the sequence coverage, i.e. a 10 times dilution of the virus in the plant homogenate resulting in approx. 10 times less viral-sequence reads. As the threshold is set at 10 times read coverage to obtain consensus sequences, no coverage was obtained by de novo assembly at dilution 10^6 and 10^7. At a 10^4 dilution, (near) complete ToBRFV genomes were recovered and at 10^5 partial (fragmented) genomes were obtained. For subsequent virus species -host combinations, the LOD was calculated based on the hypothetical |

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| | dilution at which (near) complete genomes could still be obtained . |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | The HTS test was successfully applied for the following virus/viroid host combinations, including but not limited to: 1. Citrus tristeza virus (Closterovirus) in Citrus confirmed with ELISA CTV + 2. Cotton leaf curl Gezira virus (Begomovirus) in Lavatera confirmed with RT-PCR-Sequencing generic Begomovirus + 3. Cucumber green mottle mosaic virus (Tobamovirus) in Cucumis sativus confirmed with ELISA CGMMV + 4. Cucumber mosaic virus (Cucumovirus) in Buddleja davidii confirmed with Bioassay P1++, bent-+, Wb+- 5. Cucumber mosaic virus (Cucumovirus) in Capsicum sp. confirmed with ELISA CMV + 6. Potato virus Y - O (Potyvirus) in Capsicum sp. confirmed with ELISA PVY + 7. Tomato chlorotic spot virus (Orthotospovirus) in Capsicum sp. confirmed with RT-PCR-Sequencing generic orthotospovirus TCSV+ 8. Strawberry latent ringspot virus (Stralarivirus) in Rubus idaeus confirmed with ELISA SLRSV + 9. Tobacco ringspot virus (Nepovirus) in Rosmarinus confirmed with ELISA TRSV + 10. Cherry leafroll virus (Nepovirus) in Sambucus nigra confirmed with ELISA CLRV + 11. Pepino mosaic virus (Potexvirus) in Solanum lycopersicum confirmed with ELISA PepMV + 12. Tomato brown rugose fruit virus (Tobamovirus) in Solanum lycopersicum confirmed with real-time RT-PCR specific ToBRFV + 13. Bean yellow mosaic virus (Potyvirus) in Vicia faba confirmed with RT-PCR-Sequencing generic potyvirus + |
| **Specificity value** | |
| **Analytical specificity - exclusivity** | |
| **Number of non-target organisms tested** | Not relevant for this test |
| **Specificity value** | |
| **Reproducibility** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | The repeatability and reproducibility of the test was investigated with biological material. From each dilution 10^2-10^5 three identical plant homogenate subsamples were made. RNA extraction of two of those subsamples was performed by one person at the same moment and the RNA was sequenced in the same batch (repeatability). The RNA of the third subsample was extracted by another person and sequenced at a different moment. The obtained sequence data was analysed by three qualified assessors independently. At low and medium dilutions (10^2-10^4) the ToBRFV genome was assembled in a single contiguous sequence representing the (near) complete genome with a sequence length between 6379-6353 nt and 100 % identical sequence. |

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| **Repeatability** | |
| **Provide the calculated % of agreement for a given level of the pest (see PM 7/98)** | The repeatability and reproducibility of the test was investigated with biological material. From each dilution 10^2-10^5 three identical plant homogenate subsamples were made. RNA extraction of two of those subsamples was performed by one person at the same moment and the RNA was sequenced in the same batch (repeatability). The RNA of the third subsample was extracted by another person and sequenced at a different moment. The obtained sequence data was analysed by three qualified assessors independently. At low and medium dilutions (10^2-10^4) the ToBRFV genome was assembled in a single contiguous sequence representing the (near) complete genome with a sequence length between 6379-6353 nt and 100 % identical sequence. |
| **Organism 29.:** | **Crinivirus flavisolani(PYVV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.4x10^2. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 30.:** | **Begomovirus solanumaureivariati(TOGMOV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.3x10^2. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 31.:** | **Tobamovirus maculatessellati(TOMMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.3x10^1 - 2.5x10^4. Based on 4 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 14 |
| **Specificity value** | 100 |
| **Organism 32.:** | **Ipomovirus lycopersici(TOMMOV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 8.6x10^2. Based on 1 isolate. |

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| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 33.:** | **Nepovirus lycopersici(TORSV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 3.3x10^2 - 7.0x10^3. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 8 |
| **Specificity value** | 100 |
| **Organism 34.:** | **Begomovirus manihotis(ACMV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.5x10^2. Based on 1 isolate(s). |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 35.:** | **Tymovirus mosandigenum(APMMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.3x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 36.:** | **Alfamovirus AMV(AMV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 8 |
| **Specificity value** | 100 |
| **Organism 37.:** | **Comovirus andesense(APMOV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.7x10^2. Based on 1 isolate. |

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| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 38.:** | **Nepovirus arabis(ARMV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.3x10^2 - 2.7x10^3. Based on 4 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 7 |
| **Specificity value** | 100 |
| **Organism 39.:** | **Begomovirus birdi(BGYMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.1x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 40.:** | **Tobamovirus viridimaculae(CGMMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.6x10^4 - 2.3x10^4. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 25 |
| **Specificity value** | 100 |
| **Organism 41.:** | **Polerovirus CABYV(CABYV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 5.8x10^0 - 9.9x10^2. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 34 |
| **Specificity value** | 100 |
| **Organism 42.:** | **Grablovirus vitis(GRBAV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.4x10^1. Based on 1 isolate. |

| | |
|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 43.:** | **Tymovirus latandigenum(APLV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution rate: 1.0x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 44.:** | **Cheravirus arracaciae(AVB000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.8x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 45.:** | **Apple fruit crinkle viroid(AFCVD0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.2x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 46.:** | **Ilarvirus APLPV(APLPV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution rate range: 1.2x10^1 - 6.1x10^2. Based on 5 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 18 |
| **Specificity value** | 100 |
| **Organism 47.:** | **Curtovirus betae(BCTV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 9.6x10^1. Based on 1 isolate. |

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|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 48.:** | **Benyvirus necrobetae(BNYVV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.3x10^4. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 49.:** | **Nepovirus myrtilli(BLMOV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.5x10^1 - 1.0x10^2. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 50.:** | **Begomovirus chayotis(CHAYMV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 4.2x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 51.:** | **Cheravirus avii(CRLV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 5.4x10^2 - 1.7x10^3. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 52.:** | **Robigovirus robigomaculae(CRMAV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.5x10^3. Based on 1 isolate. |

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|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 53.:** | **Robigovirus tortifoliae(CTLAV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.4x10^2. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 100 |
| **Specificity value** | 1 |
| **Organism 54.:** | **Begomovirus chillicapsici(CHILCU)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.5x10^1 - 1.0x10^2. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 4 |
| **Specificity value** | 100 |
| **Organism 55.:** | **Orthotospovirus chrysanthinecrocaulis(CSNV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 4.3x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 56.:** | **Closterovirus tristezae(CTV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 9.4x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 10 |
| **Specificity value** | 100 |
| **Organism 57.:** | **Begomovirus gossypigeziraense(CLCUGV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 5.6x10^0 - 1.2x10^1. Based on 2 isolates. |

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|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 19 |
| **Specificity value** | 100 |
| **Organism 58.:** | **Cucumovirus CMV(CMV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.1x10^2 - 3.1x10^4. Based on 5 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 41 |
| **Specificity value** | 100 |
| **Organism 59.:** | **Begomovirus macrotylomae(HYVV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 0.91. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 3 |
| **Specificity value** | 100 |
| **Organism 60.:** | **Begomovirus caricaguandongense(PALCGV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 0.93. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 3 |
| **Specificity value** | 100 |
| **Organism 61.:** | **Begomovirus capsicumhuastecoense(PHYVV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 4.0x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 62.:** | **Potexvirus marmoraucuba(PAMV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 9.8x10^2. Based on 1 isolate. |

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|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 2 |
| **Specificity value** | 100 |
| **Organism 63.:** | **Pospiviroid fusituberis(PSTVD0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.8x10^1. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 13 |
| **Specificity value** | 100 |
| **Organism 64.:** | **Nepovirus betasolani(PVB000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.8x10^1. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 6 |
| **Specificity value** | 100 |
| **Organism 65.:** | **Carlavirus pisolani(PVP000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.5x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 66.:** | **Alphanucleorhabdovirus tuberosum(PYDV00)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 3.4x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 67.:** | **Sadwavirus citri(SDV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.9x10^1. Based on 1 isolate. |

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|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 68.:** | **Stralarivirus fragariae(SLRSV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 1.6x10^1 - 2.1x10^2. Based on 3 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 14 |
| **Specificity value** | 100 |
| **Organism 69.:** | **Ipomovirus lenisbatatae(SPMMV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 6.9x10^2. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 70.:** | **Tomato chocolàte virus(TOCHV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.3x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 71.:** | **Begomovirus solanumdepravationis(TOLDEV)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.0x10^0. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 72.:** | **Torradovirus marchitezum(TOANV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.4x10^2. Based on 1 isolate. |

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|---|---|
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 73.:** | **Begomovirus citrulli(WMCSV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 2.6x10^3. Based on 1 isolate. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Organism 74.:** | **Begomovirus cocciniae(CMTNV0)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 1.5x10^1 |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100% |
| **Cross-reacts with** | |
| **Organism 75.:** | **Cherry rosette virus(CRV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 8.9x10^1 - 1.2x10^2. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 4 |
| **Specificity value** | 100% |
| **Cross-reacts with** | |
| **Organism 76.:** | **Potyvirus vetuberosi(PVV000)** |
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution range: 2.3x10^2 - 2.3x10^3. Based on 2 isolates. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 8 |
| **Specificity value** | 100 |
| **Cross-reacts with** | |
| **Organism 77.:** | **Orthotospovirus citrullomaculosi(WMSMOV)** |

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|---|---|
| **Analytical sensitivity** | |
| **What is the smallest amount of target that can be detected reliably?** | Relative dilution: 4.5x10^3. |
| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | 1 |
| **Specificity value** | 100 |
| **Cross-reacts with** | |
| **Test performance study** | |
| **Test performance study?** | no |
| | |
| The following complementary files are available online: | • Validation report VirDisc<br>• Validation report VirDisc - Appendices<br>• Additional analyses Analytical Sensitivity<br>• Additional validation data analytical sensitivity and specificity<br>• Additional validation data analytical sensitivity and specificity annex |

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