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.

Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health 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
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

Description of the test

Organism(s)	Cocadviroid cadangi (CCCVD0) Cowpea mild mottle virus / Carlavirus vignae (CPMMV0) Lettuce infectious yellows virus / Crinivirus lactucaflavi (LIYV00) Peach mosaic virus / Trichovirus persicae (PCMV00) Dichorhavirus orchidaceae (OFV000) Peach rosette mosaic virus / Nepovirus persicae (PRMV00) Potato black ringspot virus / Nepovirus solani (PBRSV0) Pepino mosaic virus / Potexvirus pepini (PEPMV0) Potato yellowing virus (PYV000) Ilarvirus SNSV (SNSV00) Physostegia chlorotic mottle virus / Alphanucleorhabdovirus physostegiae (PHCMOV) Potato virus X / Potexvirus ecspotati (PVX000) Potato virus Y / Potyvirus yituberosi (PVY000) Sri Lankan cassava mosaic virus / Begomovirus stanleyi (SLCMV0) Potato leafroll virus / Polerovirus PLRV (PLRV00)

Detete view A (Deterview et al. and a (D) (A 000)
Potato virus A / Potyvirus atuberosi (PVA000)
Emaravirus rosae (RRV000)
Crinivirus ipomeae (SPCSV0)
Carlavirus chisolani (PVH000)
Potato virus T / Tepovirus tafsolani (PVT000)
Tobacco ringspot virus / Nepovirus nicotianae
(11.5V00) Dotato virus M / Carlavirus misolani (DV/M000)
Polato virus M / Canavirus filisolarii (PVM000)
Potato virus S / Cariavirus sigmasolani (PVSUUU)
Squash vein yellowing virus / Ipomovirus
cucurbitavenaflavi (SQVYVX)
Tomato brown rugose fruit virus / Tobamovirus
fructirugosum (TOBRFV)
Tomato leaf curl New Delhi virus / Begomovirus
solanumdelhiense (TOLCND)
Tomato vellow leaf curl Thailand virus /
Pogomovirus solonumflovusthailandonsa (TVLCTH)
Potato yellow veln virus / Crinivirus flavisolani (PYVV00)
Tomato golden mottle virus / Begomovirus
solanumaureivariati (TOGMOV)
Tomato mottle mosaic virus / Tobamovirus
maculatessellati (TOMMV0)
Tomato mild mottle virus / Inomovirus lycopersici
(TOMMOV)
Tomato ringenot virue / Nonovirue luconorsici
$(T \cap RSVO)$
African cassava mosaic virus / Regomovirus
manihotis (ACMV00)
Andean potato mild mosaic virus / Tymovirus
Andean polato mila mosaic virus / Tymovirus
Altamovirus AMV (AMVUUU)
Andean potato mottle virus / Comovirus andesense (APMOV0)
Arabis mosaic virus / Nepovirus arabis (ARMV00)
Bean golden vellow mosaic virus / Begomovirus
birdi (BGYM)/0)
Cucumber green mottle messic virus / Tehemovirus
viridimaculae (CCMMV(2)
Cucurdit aphia-dorne yellows virus / Polerovirus
CABAA (CABAA0)
Grablovirus vitis (GRBAV0)
Andean potato latent virus / Tymovirus
latandigenum (APLV00)
Arracacha virus B / Cheravirus arracaciae (AVB000)
Apple fruit crinkle viroid (AFCVD0)
American plum line pattern virus / Ilarvirus APLPV
Beet curiy top virus / Curtovirus betae (BCTV00)
Beet necrotic yellow vein virus / Benyvirus
necrobetae (BNYVV0)
Blueberry leaf mottle virus / Nepovirus myrtilli
(BLMOV0)
Chavote vellow mosaic virus / Begomovirus
chavotis (CHAYMV)
Cherry rasp leaf virus / Cheravirus avii (CPLV00)
Cherry raspilear virus / Cheravirus avir (CRLVUU)
KODIGOVIRUS TORTITOIIAE (CTLAVU)
Chilli leat curl virus / Begomovirus chillicapsici
I I

	(CHILCU)
	Chrysanthemum stem necrosis virus /
	Orthotospovirus chrysanthinecrocaulis (CSNV00)
	Citrus tristeza virus / Closterovirus tristezae
	Cotton leaf curl Gezira virus / Begomovirus
	gossypigeziraense (CLCUGV)
	Cucumber mosaic virus / Cucumovirus CMV
	(CMV000)
	Honeysuckle yellow vein virus / Begomovirus
	Papava leaf curl Guandong virus / Begomovirus
	caricaguandongense (PALCGV)
	Pepper huasteco yellow vein virus / Begomovirus
	capsicumhuastecoense (PHYVV0)
	Potato aucuba mosaic virus / Potexvirus
	Potato spindle tuber viroid / Pospiviroid fusituberis
	(PSTVD0)
	Nepovirus betasolani (PVB000)
	Potato virus P / Carlavirus pisolani (PVP000)
	potato villus v / Potyvillus veluberosi (PVV000)
	tuberosum (PYDV00)
	Satsuma dwarf virus / Sadwavirus citri (SDV000)
	Strawberry latent ringspot virus / Stralarivirus
	fragariae (SLRSVU)
	Tomato chocolàte virus (TOCHV0)
	Tomato leaf deformation virus / Begomovirus
	solanumdepravationis (TOLDEV)
	Tomato marchitez virus / Torradovirus marchitezum
	(TOANVO) Watermelon chlorotic stunt virus / Begomovirus
	citrulli (WMCSV0)
Detection / identification	detection and identification
Method(s)	Molecular HTS
Mathada Malagular UTC	
Method: Molecular HIS	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the	yes
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 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 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 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 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		
Number of strains/populations of target organisms tested	7	
Specificity value	100	
Organism 5.:	Dichorhavirus orchidaceae(OFV000)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	Relative dilution range: $2.1 \times 10^3 - 2.3 \times 10^3$. Based on 2 isolates.	
Analytical specificity - inclusivity		
Number of strains/populations of target	2	

organisms tested	
Specificity value	100
Organism 6.:	Nepovirus persicae(PRMV00)
Analytical sensitivity	
What is 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 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 smallest amount of target that can be detected reliably?	Relative dilution range: $1.5 \times 10^3 - 6.8 \times 10^3$. 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 smallest amount of target that can be detected reliably?	Relative dilution range: $1.3 \times 10^3 - 5.1 \times 10^3$. Based on 2 isolates.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	7
Specificity value	100
Organism 10.:	llarvirus SNSV(SNSV00)
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Relative dilution range: $2.1 \times 10^3 - 4.2 \times 10^3$. 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 smallest amount of target that can be detected reliably?	Relative dilution range: $1.5 \times 10^2 - 3.3 \times 10^4$. 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 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 smallest amount of target that can be detected reliably?	Relative dilution range: $1.4 \times 10^2 - 8.9 \times 10^3$. 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 smallest amount of target that can be detected reliably?	Relative dilution: 8.1x10^1. Based on 1 isolate.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	1
Specificity value	100
Organism 15.:	Polerovirus PLRV(PLRV00)
Analytical sensitivity	
What is 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 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 smallest amount of target that can be detected reliably?	Relative dilution range: $3.5 \times 10^{1} - 6.7 \times 10^{2}$. 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 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 smallest amount of target that can be detected reliably?	Relative dilution: 1.1×10^4 . Based on 1 isolate.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	1	
Specificity value	100	
Organism 20.:	Tepovirus tafsolani(PVT000)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	Relative dilution range: $7.0 \times 10^{1} - 5.1 \times 10^{2}$. 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 smallest amount of target that can be detected reliably?	Relative dilution range: $2.1 \times 10^2 - 9.0 \times 10^3$. 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 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 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 smallest amount of target that can be detected reliably?	Relative dilution: 6.8x10 ³ . Based on 1 isolate.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	1	
Specificity value	100	
Organism 25.:	Tobamovirus fructirugosum(TOBRFV)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	Relative dilution range: $9.1 \times 10^3 - 1.0 \times 10^5$. 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 smallest amount of target that can be detected reliably?	Relative dilution range: $8.3 \times 10^2 - 5.0 \times 10^3$. 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 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 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 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	I
Number of non-target organisms tested	Not relevant for this test
Specificity value	
Reproducibility	1
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.
<u>Repeatability</u>	
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 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 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 smallest amount of target that can be detected reliably?	Relative dilution range: $1.3 \times 10^{1} - 2.5 \times 10^{4}$. 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 smallest amount of target that can be detected reliably?	Relative dilution: 8.6x10 ² . Based on 1 isolate.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	2	
Specificity value	100	
Organism 33.:	Nepovirus lycopersici(TORSV0)	
Analytical sensitivity	Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Relative dilution range: $3.3 \times 10^2 - 7.0 \times 10^3$. 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 smallest amount of target that can be detected reliably?	Relative dilution: 3.5×10^2 . 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 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	
Analytical sensitivity	
<u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably?	Relative dilution range: 6.2x10 ³ - 4.5x10 ⁴ . Based on 3 isolate(s).
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity	Relative dilution range: 6.2x10 ³ - 4.5x10 ⁴ . Based on 3 isolate(s).
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested	Relative dilution range: 6.2x10 ³ - 4.5x10 ⁴ . Based on 3 isolate(s). 8
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.:	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOV0)
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOV0)
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably?	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOV0) Relative dilution: 1.7x10^2. Based on 1 isolate.
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOV0) Relative dilution: 1.7x10^2. Based on 1 isolate.
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target	Relative dilution range: 6.2×10^3 - 4.5×10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOVO) Relative dilution: 1.7×10^2. Based on 1 isolate. 1
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value	Relative dilution range: 6.2×10^3 - 4.5×10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOVO) Relative dilution: 1.7×10^2. Based on 1 isolate. 1 100
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target Specificity value Organisms tested Specificity value	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOVO) Relative dilution: 1.7x10^2. Based on 1 isolate. 1 100 Nepovirus arabis(ARMVO0)
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 38.: Analytical sensitivity	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOV0) Relative dilution: 1.7x10^2. Based on 1 isolate. 1 100 Repovirus arabis(ARMV00)
Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 37.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 38.: Analytical sensitivity What is smallest amount of target that can be detected reliably?	Relative dilution range: 6.2x10^3 - 4.5x10^4. Based on 3 isolate(s). 8 100 Comovirus andesense(APMOVO) Relative dilution: 1.7x10^2. Based on 1 isolate. 1 100 Nepovirus arabis(ARMV00) Relative dilution range: 1.3x10^2 - 2.7x10^3. Based on 4 isolates.

Number of strains/populations of target organisms tested	7	
Specificity value	100	
Organism 39.:	Begomovirus birdi(BGYMV0)	
Analytical sensitivity		
What is 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 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	Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	Relative dilution range: $5.8 \times 10^{0} - 9.9 \times 10^{2}$. 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 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	
Specificity value Organism 43.:	100 Tymovirus latandigenum(APLV00)	
Specificity value Organism 43.: <u>Analytical sensitivity</u>	100 Tymovirus latandigenum(APLV00)	
Specificity value Organism 43.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably?	100 Tymovirus latandigenum(APLV00) Relative dilution rate: 1.0x10^3. Based on 1 isolate.	

Number of strains/populations of target organisms tested	2
Specificity value	100
Organism 44.:	Cheravirus arracaciae(AVB000)
Analytical sensitivity	
What is 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 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.:	llarvirus APLPV(APLPV0)
Analytical sensitivity	
What is 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 smallest amount of target that can be detected reliably?	Relative dilution: 9.6x10^1. Based on 1 isolate.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	1
Number of strains/populations of target organisms tested Specificity value	1 100
Number of strains/populations of target organisms tested Specificity value Organism 48.:	1 100 Benyvirus necrobetae(BNYVV0)
Number of strains/populations of target organisms tested Specificity value Organism 48.: <u>Analytical sensitivity</u>	1 100 Benyvirus necrobetae(BNYVV0)
Number of strains/populations of target organisms tested Specificity value Organism 48.: Analytical sensitivity What is smallest amount of target that can be detected reliably?	1 100 Benyvirus necrobetae(BNYVV0) Relative dilution: 3.3x10^4. Based on 1 isolate.

Number of strains/populations of target organisms tested	1	
Specificity value	100	
Organism 49.:	Nepovirus myrtilli(BLMOV0)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	Relative dilution range: $2.5 \times 10^{1} - 1.0 \times 10^{2}$. 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 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 smallest amount of target that can be detected reliably?	Relative dilution range: $5.4 \times 10^2 - 1.7 \times 10^3$. 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 smallest amount of target that can be detected reliably?	Relative dilution: 1.5×10^3 . Based on 1 isolate.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	1	
Specificity value	100	
Organism 53.:	Robigovirus tortifoliae(CTLAV0)	
Analytical sensitivity	Analytical sensitivity	
What is 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 smallest amount of target that can be detected reliably?	Relative dilution range: $2.5 \times 10^{1} - 1.0 \times 10^{2}$. 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 smallest amount of target that can be detected reliably?	Relative dilution: 4.3×10^3 . 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 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 smallest amount of target that can be detected reliably?	Relative dilution range: 5.6x10^0 - 1.2x10^1. Based on 2 isolates.
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	19
Specificity value	100
Organism 58.:	Cucumovirus CMV(CMV000)
Analytical sensitivity	
What is smallest amount of target that can be	Deletive dilution remain $1.1 \times 1002 = 2.1 \times 1004$
detected reliably?	Based on 5 isolates.

Number of strains/populations of target organisms tested	41
Specificity value	100
Organism 59.:	Begomovirus macrotylomae(HYVV00)
Analytical sensitivity	
What is 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 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 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
organishis tested	
Specificity value	100
Specificity value Organism 62.:	100 Potexvirus marmoraucuba(PAMV00)
Specificity value Organism 62.: <u>Analytical sensitivity</u>	100 Potexvirus marmoraucuba(PAMV00)
Specificity value Organism 62.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably?	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate.
Specificity value Organism 62.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably? <u>Analytical specificity - inclusivity</u>	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate.
Specificity value Organism 62.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate. 2
Specificity value Organism 62.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested Specificity value	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate. 2 100
Specificity value Organism 62.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested Specificity value Organism 63.:	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate. 2 100 Pospiviroid fusituberis(PSTVD0)
Specificity value Organism 62.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 63.: Analytical sensitivity	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate. 2 100 Pospiviroid fusituberis(PSTVD0)
Specificity value Organism 62.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 63.: Analytical sensitivity What is smallest amount of target that can be detected reliably?	100 Potexvirus marmoraucuba(PAMV00) Relative dilution: 9.8x10^2. Based on 1 isolate. 2 100 Pospiviroid fusituberis(PSTVD0) Relative dilution: 1.8x10^1. Based on 1 isolate.

Number of strains/populations of target organisms tested	13	
Specificity value	100	
Organism 64.:	Nepovirus betasolani(PVB000)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	Relative dilution: 6.8x10^1. Based on 1 isolate.	
Analytical specificity - inclusivity	Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	6	
Specificity value	100	
Organism 65.:	Carlavirus pisolani(PVP000)	
Analytical sensitivity		
What is smallest amount of target that can be detected reliably?	Relative dilution: 6.5×10^3 . Based on 1 isolate.	
Analytical specificity - inclusivity		
Number of strains/populations of target organisms tested	1	
Specificity value	100	
Organism 66.:	Potyvirus vetuberosi(PVV000)	
Analytical sensitivity		
What is smallest amount of target that can be	Relative dilution range: 2.3x10^2 - 2.3x10^3.	
detected reliably?	Based off 2 Isolates.	
detected reliably? Analytical specificity - inclusivity	Based off 2 Isolates.	
detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested	8	
detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested Specificity value	8 100	
detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested Specificity value Organism 67.:	8 100 Alphanucleorhabdovirus tuberosum(PYDV00)	
detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested Specificity value Organism 67.: <u>Analytical sensitivity</u>	8 100 Alphanucleorhabdovirus tuberosum(PYDV00)	
detected reliably? <u>Analytical specificity - inclusivity</u> Number of strains/populations of target organisms tested Specificity value Organism 67.: <u>Analytical sensitivity</u> What is smallest amount of target that can be detected reliably?	8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate.	
detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 67.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity	8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate.	
detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 67.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested	8 8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate. 1	
detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 67.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value	8 8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate. 1 100	
detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 67.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 68.:	8 8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate. 1 1 100 Sadwavirus citri(SDV000)	
detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 67.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 68.: Analytical sensitivity	8 8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate. 1 100 Sadwavirus citri(SDV000)	
detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 67.: Analytical sensitivity What is smallest amount of target that can be detected reliably? Analytical specificity - inclusivity Number of strains/populations of target organisms tested Specificity value Organism 68.: Analytical sensitivity What is smallest amount of target that can be detected reliably?	8 8 100 Alphanucleorhabdovirus tuberosum(PYDV00) Relative dilution: 3.4x10^3. Based on 1 isolate. 1 100 Sadwavirus citri(SDV000) Relative dilution: 6.9x10^1. Based on 1 isolate.	

Number of strains/populations of target organisms tested	1
Specificity value	100
Organism 69.:	Stralarivirus fragariae(SLRSV0)
Analytical sensitivity	
What is 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 70.:	Ipomovirus lenisbatatae(SPMMV0)
Analytical sensitivity	
What is 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 71.:	Tomato chocolàte virus(TOCHV0)
Analytical sensitivity	
What is 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 72.:	Begomovirus solanumdepravationis(TOLDEV)
Analytical sensitivity	
What is 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 73.:	Torradovirus marchitezum(TOANV0)
Analytical sensitivity	
What is 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	1
Specificity value	100
Organism 74.:	Begomovirus citrulli(WMCSV0)
Analytical sensitivity	
What is 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
Test performance study	
Test performance study?	no
The following complementary files are available online:	 Validation report VirDisc Validation report VirDisc - Appendices Additional analyses Analytical Specificity Additional analyses Analytical Sensitivity Additional analyses Analytical Sensitivity and Specificity - Appendices

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