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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 3/21(3)

Phytosanitary Procedures

PM 3/21(3) Post entry quarantine for potato

Specific scope

This Standard describes inspection and tests for the detection of pests (bacteria, viroids and viruses) infecting *Solanum* species or hybrids imported for germplasm conservation, breeding or research purposes, in post-entry quarantine. It satisfies the requirements of EPPO Standard PM 8/1 *Commodity-specific phytosanitary measures for potato*.

Specific approval and amendments

First approved in 1983–09.
Revised in 2004–09 (with editorial corrections in 2006–03).
Second revision approved in 2019–09.

Introduction

This phytosanitary procedure for inspection and testing potato in post-entry quarantine should be used by NPPOs in order to prevent the entry and spread of quarantine pests of potato into and within the EPPO region as recommended in EPPO Standard PM 8/1 *Commodity -specific phytosanitary measures for potato*. In addition, the procedure is designed to detect regulated non-quarantine pests of potato and most potato infecting bacteria, viroids and viruses.[1] In principle, infected material should not be released from post-entry quarantine since it may be used for planting for seed potato production and for field trials. However, countries may lay down special measures for release from quarantine of infected or not fully tested material, for example for use under confinement, depending on the pest and the purpose.

The Standard takes into account recommendations made in FAO/IPGRI Guidelines for Potato (Jeffries, 1998), previous EPPO documents on testing in post-entry quarantine (EPPO, 1984; EPPO/CABI, 1996) and methods used by various EPPO member countries.

The procedure should be applied to potato breeding material: *Solanum tuberosum* and other cultivated *Solanum* spp., wild stolon- and tuber-forming *Solanum* spp., and closely related *Solanum* spp. that hybridize with potato and may not develop stolons or tubers. It covers both vegetative material and true potato seeds.

Specific definitions

Accession: a sample of seeds with a unique gene bank accession number.
Candidate material: material received for testing under quarantine, i.e. tubers, microplants, true potato seeds.
Complete vegetative cycle: the cycle of growth from seed or microplant through to mature plant and the natural onset of senescence.
Line: a cultivar (clone).
Microplants of potato: plants *in vitro* (including micro-tubers) of tuber-forming *Solanum* spp.
Unit: a single microplant, single tuber or single true potato seed.
Vegetative material: material submitted for testing under quarantine in the form of tubers or plants (including microplants).

Outline of the procedure

The phytosanitary procedure involves the following:
• Establishing candidate material *in vitro* or *in vivo*.[2]

[1]Virus names in italics are of species approved by the International Committee on Taxonomy of Viruses as of 20/12/2018 https://talk.ictvonline.org/taxonomy/vmr/m/vmr-file-repository/8011. Those not in italics have not yet been approved.

[2]EPPO recommends *in vitro* propagation as a phytosanitary confinement procedure for potato quarantine. This complements the recommendations of EPPO Standard PM 4/28 *Certification scheme for seed potatoes* (EPPO, 1999) and is in line with the recommendations of the IPGRI. However, *in vivo* quarantine procedures that give equivalent phytosanitary security may also be used. An example of such a procedure is described in Appendix 2.

- Growing candidate material (and plants derived from it) under quarantine[3] /confinement[4] (e.g. *in vitro* or in insect-proof glasshouses or growth rooms.)[5].
- Testing each unit of the candidate material (or plants derived from it) for pests using the tests indicated in this Standard, at a stage of plant growth which optimizes detection of the pest, including those pests with an uneven distribution or low concentrations.
- Growing the candidate material (or plants derived from it) in the glasshouse, usually through a complete vegetative cycle, and inspecting for symptoms of disease, with appropriate investigation.
- Growing and maintaining the tested material using procedures which minimize the risk of cross-infection or contamination and accidental release of a pest.
- Destroying material found to be infected unless, for material infected with viruses, carrying out virus elimination following FAO/IPGRI recommended procedures (Jeffries, 1998). Virus elimination should be started only after testing the microplants for freedom from at least viroids and regulated bacteria. After viruses have been eliminated, the material should be subject to the full quarantine procedure. Following FAO/IPGRI recommended procedures (Jeffries, 1998), elimination of viroids and phytoplasmas may be attempted but plants infected with other regulated bacteria, other than phytoplasmas, should be destroyed.
- Material that passes the inspection and tests described should be released from post-entry quarantine with a Germplasm Health Statement that specifies the tests done.

Requirements for growing conditions for post-entry quarantine based on *in vitro* propagation (for *in vivo* propagation, see Appendix 2)

Candidate material for post-entry quarantine may be received as tubers, microplants or true potato seed.

The recommended procedure involves establishing each unit of candidate material as a single microplant *in vitro* (the Mother Plant). The Mother Plant should be subcultured to produce microplants for testing and for growing in the glasshouse for visual inspection and further testing.

In the glasshouse, plants should be grown over a full vegetative cycle at 18–25°C and with at least a 14-h photoperiod. Plants should be slightly shaded if necessary to help symptom development.

An example of a propagation and testing programme based on *in vitro* propagation for candidate material received as microplants or tubers is described in Fig. 1 and for material received as true potato seed in Fig. 2. For more details on these propagation programmes see Appendix 1.

Requirements for inspection

On receipt:

- Microplants should be inspected for the absence of arthropod (particularly mites and thrips), bacterial and fungal pests. Infested material should normally be destroyed, although material contaminated with saprophytic endogenous bacteria may be quarantine-tested at the discretion of the NPPO.
- Tubers should be inspected for external disease symptoms and if these are present micropropagation should only be done at the discretion of the NPPO.
- True seed should be inspected for the presence of mites. If present these may be killed by storing the seeds at −20°C for 7 days.

During propagation, microplants should be inspected at regular intervals, particularly in the first 2 weeks after subculture, for bacterial and fungal contamination.

Glasshouse plants should be inspected at least once a week for symptoms of disease. At each inspection, records should be made of the plants which have been inspected and if symptoms are observed these should be recorded. Harvested tubers should be cut and inspected for disease symptoms, e.g. vascular necrosis, spraing, zebra chip.

Requirements for pathogen testing

All the tests (biological indexing, molecular tests, serological tests and others) presented in this Standard may be adjusted to suit individual laboratories and to reflect developments in techniques, provided that they are adequately validated or verified and the uncertainty of detection is not increased.

The pests for testing are divided into two tables. Pests for more intensive testing are shown in 1 and other pests for less intensive testing are shown in Table 2.

Vegetative material

For vegetative material, plants should be tested at least twice during propagation for the pests in Table 1 using two independent tests which, preferably, are based on different biological principles, one of which should be a bioassay for viruses that are mechanically transmissible. For viruses which are not mechanically transmitted, or are not reliably mechanically transmitted, another independent test should be used.

For material received as microplants, one test can be done on microplants and the other, or both test(s), on glasshouse-grown plants and progeny tubers if relevant. Material received as tubers should be tested for *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia*

[3]See ISPM 34 *Design and operation of post-entry quarantine stations for plants* (IPPC, 2016a).

[4]See EPPO Standard PM 3/64 *Intentional import of organisms that are plant pests or potential plant pests* (EPPO, 2006).

[5]Hereafter only glasshouses will be referred to in the text but growth rooms may also be used.

solanacearum and *R. syzygii* subsp. *indonesiensis*[6] (hereafter referred to as the *Ralstonia solanacearum* species complex (RSSC)). Tests on tubers (tuber sap or sprouts) for other pests are optional, but testing at this stage may reduce the risk of micropropagating infected material.

Additional viruses have been reported to infect potato or related species naturally (Jeffries & Lacomme, 2018) and these are listed in Table 2. Although most of these may be detected using bioassay, an additional test based on a different biological principle may be used to reduce the uncertainty of detection, particularly if the plant material originates from a country where these viruses are known to occur. For viruses which are not mechanically transmitted, or not reliably mechanically transmitted, another independent test should be used.

If disease symptoms are present, but none of the tests is positive, then electron microscopy following EPPO Standard PM 7/126 *Electron microscopy in diagnosis of plant viruses* and/or high-throughput sequencing (HTS), also referred to as next-generation sequencing, should be considered (Boonham *et al*., 2014; Massart *et al*., 2014).

True potato seed

For true potato seeds, plants derived from candidate material should be tested at least once for seed-transmissible viroid and virus species, i.e. *Potato spindle tuber viroid*, *Andean potato latent virus*, *Andean potato mild mosaic virus, Arracacha virus B*–oca strain, *Potato black ringspot virus, Potato virus T* and Potato yellowing virus. At least 20 seedlings per accession should be tested and released to breeders to avoid in-breeding depression and genetic drift during subsequent crossings (Hawkes, 1990).

At the end of the inspection and testing programme, the overall result should be recorded. In the case of a positive result, further testing should be done to confirm the identity of the pest, particularly if the result is likely to be challenged (see later: Administration of the procedure).

Viroids

Tests may be done on tubers, microplants or glasshouse grown plants. Sampling procedures and RNA extraction are described in ISPM 27 DP 07: *Potato spindle tuber viroid*. Testing of microplants should be done on subcultures derived from the Mother Plant with good growth (4–6 weeks old and with stems of at least 5 cm length and with full leaves). For glasshouse-grown plants (>5 cm tall) a fully expanded leaflet from each plant should be tested.

The viroid species of principal concern is *Potato spindle tuber viroid* (PSTVd; Genus *Pospiviroid*) since it was until recently the only viroid known to infect cultivated species of potato naturally. However, the viroid species *Chrysanthemum stunt viroid* (CSVd; Genus *Pospiviroid*) has now been detected in asymptomatic potato plants from Russia (Matsushita *et al*., 2019). Additionally, the viroid species *Tomato planta macho viroid* (previously *Mexican papita viroid*) has been found infecting the solanaceous weed species *Solanum cardiophyllum* (papita guera, cimantli), which is used in potato breeding (Martinez-Soriano *et al*., 1996; Thieme *et al*., 2010; Verhoeven *et al*., 2011). Furthermore, other viroid species *Citrus exocortis viroid* (Semancik *et al*., 1973; Verhoeven *et al*., 2004), *Columnea latent viroid* (Verhoeven *et al*., 2004) and *Tomato chlorotic dwarf viroid* (Singh *et al*., 1999) have been shown to infect potato experimentally. Therefore, it is recommended to use a generic molecular test for viroid (pospiviroid) detection, chosen from hybridization with a digoxigenin-labelled cRNA probe, conventional RT-PCR using the primers of Verhoeven *et al*. (2004), and real-time RT-PCR using the GenPospi test (Botermans *et al*., 2013). However, since bioassay is not recommended for detection of PSTVd in this Standard, an additional different test selected from those mentioned above, or more specific tests for CSVd and PSTVd detection such as the real-time tests of Mumford *et al*. (2000b) and Boonham *et al*. (2004), should be used (see Table 4). For CSVd these tests are described in EPPO Standard PM 7/006 and for PSTVd in ISPM 27 DP 07. Some tests are also described in EPPO Standard PM 7/033 (to be replaced by a new pospiviroid standard which is under development). Since none of the currently available tests, apart from the real-time test for CSVd, specifically identifies the viroid, for identification a conventional RT-PCR should be done and the PCR product sequenced and analysed as described in ISPM 27 DP 07 for PSTVd.

Viruses

Testing for viruses should be done on microplants (optional) and/or glasshouse-grown plants. Usually, bioassay would only be done on glasshouse-grown plants because of the relatively large quantity of sap required for inoculation. Optionally tubers (tuber sap or sprouts) may be tested but this should be done only to reduce the risk of micropropagating virus-infected material. For microplants, tests should be done on microplant subcultures derived from the Mother Plant with good growth (4–6 weeks old and with stems of at least 5 cm length). At least two microplants should be tested to reduce the uncertainty of detection because of uneven virus distribution. This is particularly important for detecting, for example, the virus species *Potato mop-top virus* (Nisbet *et al*., 2004). Cultures of each line should be

[6] *R. solanacearum* has been reclassified into three distinct species: *R. solanacearum* (Phylotype II and potato infecting), *Ralstonia pseudosolanacearum* (Phylotype I and III not potato infecting) and *Ralstonia syzygii* (Phylotype IV). *Ralstonia syzygii* comprises three subspecies, one of which is subsp. *indonesiensis* found on *Solanum tuberosum* (potato) in Indonesia (see Safni *et al*., 2014 and PM 7/21 (2) *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex)).

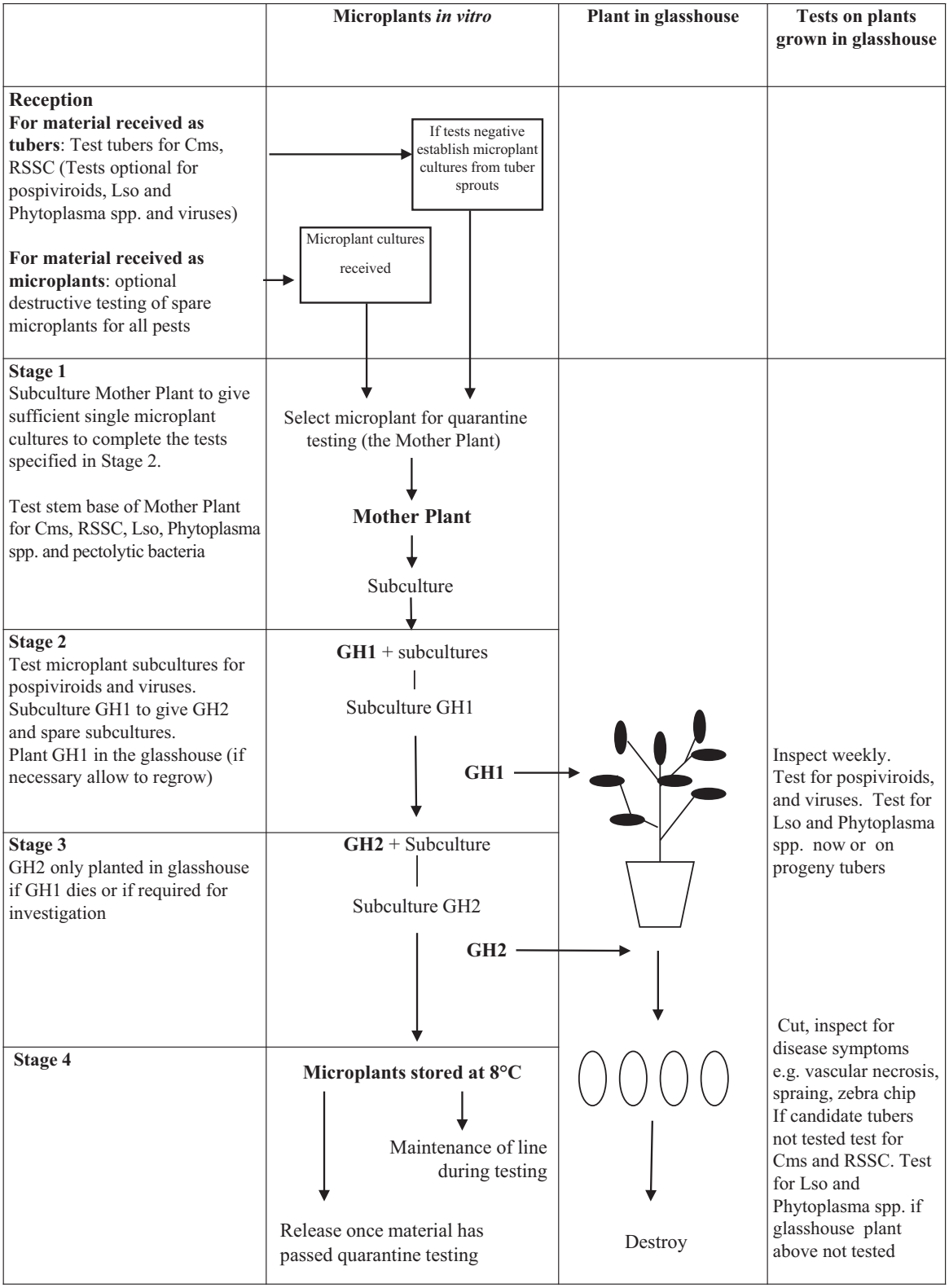

Fig. 1 Example of propagation pathway for material received as tubers or as microplants. Cms, *Clavibacter michiganensis* subsp. *sepedonicus;* RSSC, *Ralstonia solanacearum* species complex *(Ralstonia solanacearum* and *R. syzygii* subsp. *indonesiensis);* Lso, '*Candidatus* Liberibacter solanacearum'; GH, glasshouse plant.

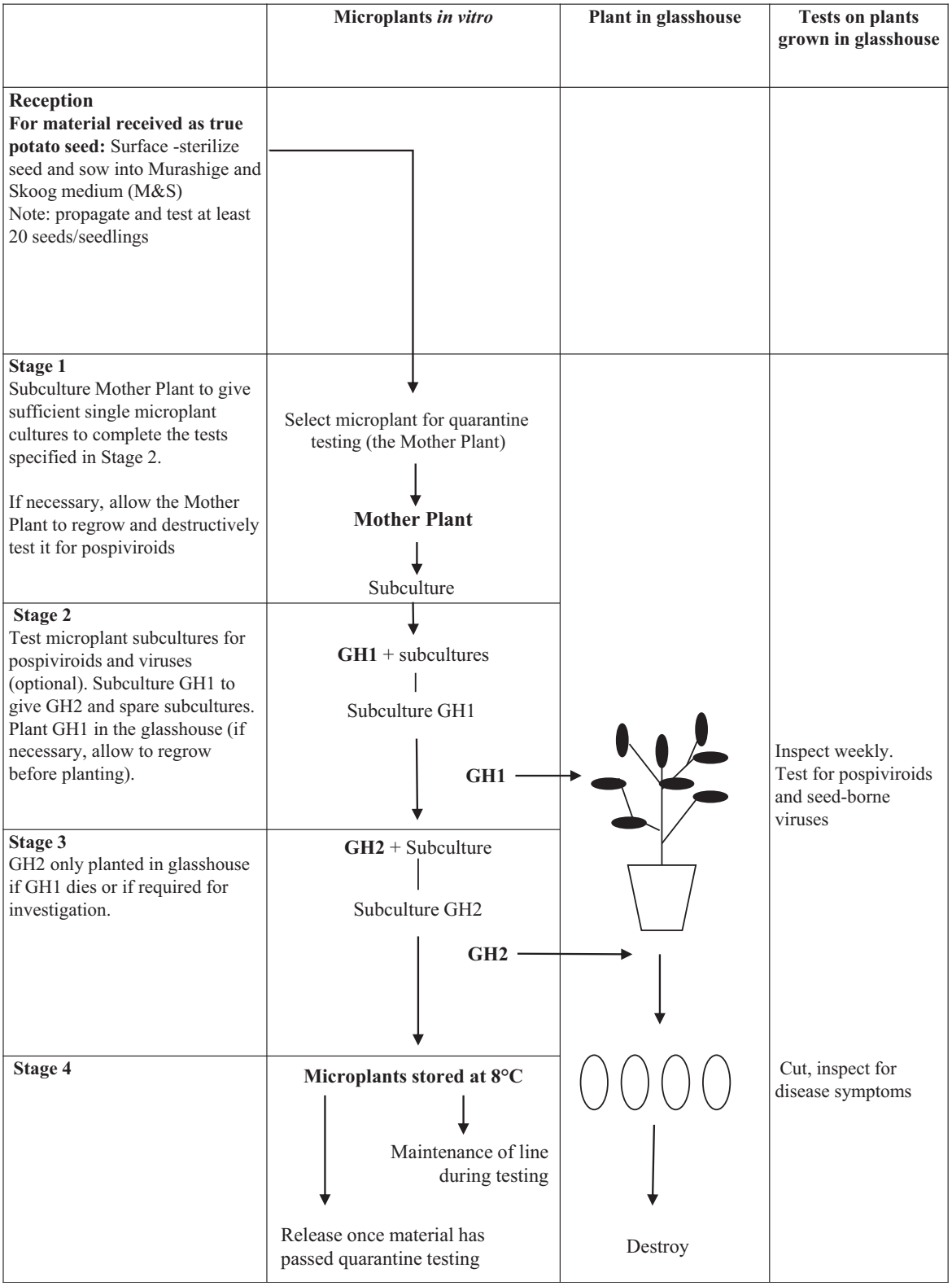

Fig. 2 Example of propagation pathway for material received as true potato seed (for each accession). Cms, *Clavibacter michiganensis* subsp. *sepedonicus*;RSSC, *Ralstonia solanacearum* species complex (*Ralstonia solanacearum* and *R. syzygii* subsp. *indonesiensis*); Lso, '*Candidatus* LiberibacterSolanacearum'; GH, glasshouse plant.

Table 1. List of pests recommended to be tested and available test method (Y = yes, N = no)

PEST	Acronym	Genus	Pest list/Quarantine pest status			Test method			Comment (and diagnostic standard if available)
			EPPO*	EU†	EAEU‡	ELISA	Bio-assay	Other	
Viroid species									
Chrysanthemum stunt viroid	CSVd	*Pospiviroid*	A2	IIA2	A1	N	N	DIG, RT-PCR	New PM 7 Standard on pospiviroids in preparation
Potato spindle tuber viroid§	PSTVd	*Pospiviroid*	A2	IIA2	A2	N	N	DIG, RT-PCR	PM 7/33 (to be replaced by a new PM 7 Standard on pospiviroids in preparation)
Tomato planta macho viroid	TPMVd	*Pospiviroid*				N	N	DIG, RT-PCR	New PM 7 Standard on pospiviroids in preparation
Virus species									
Andean potato latent virus§	APLV	*Tymovirus*	A1	IA1	A1	Y	Y	RT-PCR	Serological variability. Antibodies may detect APMMV and other related tymoviruses PM 7/132
Andean potato mild mosaic virus§	APMMV	*Tymovirus*	A1			Y	Y	RT-PCR	Formerly APLV-Hu so legislation needs to be updated to include it PM 7/132
Andean potato mottle virus	APMoV	*Comovirus*	A1	IA1	A1	Y	Y	RT-PCR	
Arracacha virus B-oca strain material	AVB-O	*Cheravirus*		IA1		Y	Y	RT-PCR	Recent molecular analysis shows that the oca strain may be a new species (Jones *et al.,* 2019)
Potato black ringspot virus§	PBRSV	*Nepovirus*	A1	IA1	A1	Y	Y	RT-PCR	
Potato latent virus	PotLV	*Carlavirus*				Y	Y	RT-PCR	Bioassay may be unreliable
Potato leafroll virus	PLRV	*Polerovirus*		IA1		Y	N	RT-PCR	Not mechanically transmitted
Potato mop-top virus	PMTV	*Pomovirus*				Y	Y	RT-PCR	Virus unevenly distributed
Potato virus A	PVA	*Potyvirus*		IA1**		Y	Y	RT-PCR	
Potato virus M	PVM	*Carlavirus*		IA1**		Y	Y	RT-PCR	
Potato virus P††	PVP	*Carlavirus*				Y	Y	RT-PCR	
Potato virus S	PVS	*Carlavirus*		IA1**		Y	Y	RT-PCR	
Potato virus T§	PVT	*Tepovirus*	A1	IA1**	A1	Y	Y	RT-PCR	
Potato virus V	PVV	*Potyvirus*		IA1**		Y	Y	RT-PCR	
Potato virus X	PVX	*Potexvirus*		IA1**		Y	Y	RT-PCR	
Potato virus Y	PVY	*Potyvirus*		IA1**		Y	Y	RT-PCR	
Potato yellow vein virus‡‡	PYVV	*Crinivirus*	A1		A1	N	N	RT-PCR	Not mechanically transmitted
Potato yellowing virus§,‡‡	PYV	*Ilarvirus*	A1		A1	Y	N	RT-PCR	Bioassay may be unreliable; isolates serologically variable
Tobacco rattle virus	TRV	*Tobravirus*				N	Y	RT-PCR	NM types not detected by ELISA and unreliably by bioassay; other isolates serologically variable
Tomato spotted wilt virus	TSWV	*Orthotospovirus*	A2	IIA2		Y	Y	RT-PCR	Polyclonal antisera may cross react with other orthotospoviruses PM 7/34 under revision

(continued)

Table 1 *(continued)*

PEST	Acronym	Genus	Pest list/Quarantine pest status			Test method			Comment (and diagnostic standard if available)
			EPPO*	EU†	EAEU‡	ELISA	Bio-assay	Other	
Bacteria									
Clavibacter michiganensis subsp. *sepedonicus*		*Clavibacter*	A2	IIA2			Y	IF, MTNA, PCR	Council Directive 93/85/EEC as amended by Commission Directive 2006/56/EC PM 7/59
Ralstonia solanacearum		*Ralstonia*	A2	IIA2	A1		Y	IF, SMSA, PCR	Council Directive 98/57EC as amended by Commission Directive 2006/63/CE PM 7/021
R. syzygii subsp. *indonesiensis*		*Ralstonia*	A1				Y	IF, SMSA, PCR	PM 7/021
'*Candidatus* Liberibacter solanacearum' Solanaceae haplotypes		Liberibacter	A1		A1			PCR	Unevenly distributed and may be at low levels ISPM 27 DP 21 EPPO PM7/in preparation
'*Ca*. Phytoplasma solani' (Stolbur)		Phytoplasma	A2	IIA2				PCR	May be unevenly distributed and at low levels ISPM 27 DP12 EPPO PM 7/133
'*Ca*. Phytoplasma americanum' (Potato purple-top wilt)		Phytoplasma	A1					PCR	May be unevenly distributed and at low levels ISPM 27 DP12 EPPO PM 7/133
Pectolytic bacteria (e.g. *Dickeya* spp., *Pectobacterium* spp.)								CVPM medium, PCR	

*EPPO A1 list https://www.eppo.int/QUARANTINE/listA1.htm; EPPO A2 list https://www.eppo.int/QUARANTINE/listA2.htm.
†EU Directive 2000/29 (EU, 2000) PLRV, PVS, PVV, PVX, PVY are non-European isolates.
‡The Eurasian Economic Union.
§For plants derived from true potato seeds, test only for these pests.
**Only non-European isolates are regulated.
††Potato rough dwarf virus is a strain of PVP (Nisbet *et al*., 2006).
‡‡Commission Directive 2008/61/EC lists PYV and potato yellow vein disease for quarantine testing.

tested separately. Plants giving a positive test result do not need to be planted in the glasshouse unless they are required for further investigation.

Glasshouse-grown plants should be tested when about 25 cm tall, at or near flowering. Samples should be taken from at least two positions on each plant (or each stem if the plant has produced lateral stems), including a young, fully expanded terminal leaflet at the top of a stem and an older terminal leaflet from a midway position. Leaves from different lines should not be 'bulked' together for ELISA unless the bulking rate has been validated. Leaves from up to five plants may be 'bulked' together for bioassay of plants from different lines.

Electron microscopy may be used to confirm the presence of virus particles. General instructions are described in EPPO Standard PM 7/126 *Electron microscopy in diagnosis of plant viruses*. Virus identity may be confirmed by (specific) (RT-)PCR, and/or sequencing and analysis of the PCR product.

ELISA

The sensitivity and specificity of ELISA depends on the antibodies used. The user should be aware of any known serotypes or strains of the virus (Jeffries, 1998) that may increase the uncertainty of detection. For many of the viruses listed in Tables 1 and 2, antibodies are available commercially, but validation data may not be readily available and therefore antibodies should be validated before use. Although in general antibodies should be used according to the manufacturers' instructions, instructions may be modified by the laboratory if the test is validated. General instructions are described in EPPO Standard PM 7/125 *ELISA tests for viruses*.

Molecular tests

For many of the viruses listed in this Standard, tests are available that will allow detection of all or most viruses in a virus genus (e.g. *Begomovirus*, *Carlavirus*, *Potexvirus*, *Potyvirus*) and examples are referenced in Appendix 3, Table 3. Examples of more specific viroid and virus tests are referenced in Appendix 3, Table 4. When using generic tests, virus identity should be confirmed by sequencing and analysis of the PCR product.

Bioassay

The bioassay procedure is described in Appendix 4. The recommended list of indicator plants (Appendix 4, Table 5) is based on the first version of this Standard, but other indicators may be used if they have been shown by the NPPO to give the same broad coverage of detection as the recommended indicators. Broad coverage is essential in order to detect unknown potato viruses which may be present.

Bacteria

For the bacteria *Clavibacter michiganensis* subsp. *sepedonicus* and the RSSC (*Ralstonia solanacearum* and *R. syzygii* subsp. *indonesiensis*), tests may be done on the tubers received (heel-end cores), the mother microplant and subsequent progeny tubers. If progeny tubers are not produced, stem segments just above soil level may be tested. Tests recommended for use in potato quarantine for *C. m.* subsp. *sepedonicus* are immunofluorescence (IF), PCR (conventional and real-time) and bioassay on aubergine (*Solanum melongena* cv. Black Beauty). Tests recommended for the RSSC are IF, SMSA selective isolation medium and PCR (conventional and real-time PCR tests). For detection of *C. michiganensis* subsp. *sepedonicus* and the RSSC, two different independent tests based on different biological principles should be used. If either test gives a positive result, bacterial identity should be confirmed by sequencing the PCR product and if required the diagnosis completed by isolation and identification tests and pathogenicity testing of the pure culture according to relevant EPPO Standards or EU Directives.[7] If bioassay is done as one of the tests and no symptoms are observed after 4 weeks, further testing should be done on a composite sample of 1-cm stem sections of each test plant taken above the inoculation site using IF, SMSA medium or PCR as relevant. Although test methods have been validated on a composite sample size of 200 potato tubers or stems from potato and other plant pieces, they may be applied to samples with fewer numbers (see EPPO standards PM7/21 and PM 7/59), such as those found in post-entry quarantine.

For *'Ca.* Phytoplasma solani' (Potato stolbur), *'Ca.* Phytoplasma americanum' (Potato purple-top wilt) and '*Ca.* Liberibacter solanacearum' there is uncertainty about when is the most appropriate time to test because the bacteria may be unevenly distributed and at low concentrations. DNA extracted from microplants (the Mother Plant) should be tested and also DNA extracted from tubers (heel-ends) on receipt or from progeny tubers (produced from glasshouse grown plants), or from leaf midribs of glasshouse-grown plants, when about 25 cm tall, but prior to flowering and pollen production.

Phytoplasmas should be tested for by using universal phytoplasmas primers. ISPM 27 DP12: *Phytoplasmas* and EPPO Standard PM 7/133 *Generic detection of phytoplasmas* provide details of nucleic acid extraction and universal conventional nested-PCR and real-time PCR tests. However, false positives with bacterial species (e.g. *Paenabacillus* spp.) closely related to phytoplasmas may occur with the PCR tests recommended in this ISPM. For more specific detection universal real-time tests using MGB modified (Malandraki *et al*., 2015) or LNA modified (Palmano *et al*., 2015) probes may be used. Preferably two different tests should be used. If positives are found they should be confirmed by retesting the samples using nested-PCR and sequencing the product, or HTS.

'*Ca.* Liberibacter solanacearum' should be tested using the real-time PCR tests of Li *et al*. (2009) and Teresani *et al*. (2014) as described in ISPM 27 DP 21: *Candidatus Liberibacter solanacearum* and PM7/in preparation, respectively. Preferably both these tests should be used. If positives are found they should be confirmed by retesting the samples using conventional PCR as described in ISPM 27 DP 21 and PM7/in preparation, and sequencing the product. If required the haplotype may be determined as described in ISPM 27 DP 21 and PM7/in preparation.

Molecular tests for bacteria are listed in Appendix 5.

Quality control

Before first use tests should preferably be validated or verified according to PM 7/98: *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*. Appropriate controls should be used for all tests (for ELISA see PM 7/125: *ELISA tests for viruses*, for molecular see ISPM 27 DP 07: *Potato spindle tuber viroid*, for bioassay more general controls can be used as described by Roenhorst *et al*., 2013).

Wherever possible the (RT-)PCR tests should be multiplexed with internal endogenous control primers (and

[7]For *C. m. sepedonicus* all detection tests mentioned in this Standard (except real-time PCR) are described in Council Directive 93/85/EEC as amended by Commission Directive 2006/56/EC (EU, 2006a) and/or in EPPO Standard PM7/42. For *R. solanacearum* all detection tests mentioned in this Standard are described in Council Directive 98/57EC as amended by Commission Directive 2006/63/CE (EU, 2006b) or EPPO Standard PM7/21. More novel methods, especially new real-time PCR methods will be included when these Directives are revised. The use of the two test methods is different in this post-entry quarantine procedure and in the EU Directives. For post-entry quarantine, use of two test methods could (in some cases) increase stringency of the testing, whereas in the EU Directives use of two test methods reduces the number of false positives.

Table 2. Other viruses reported to infect potato naturally (based on Jeffries & Lacomme, 2018) and indication of whether or not they can be detected by bioassay (Y = yes, N = no, ? = Reliability of bioassay needs establishing).

Virus species	Acronym	Genus	Quarantine status			Bio-assay	Comment (and diagnostic standard if available)
			EPPO	EU	EAEU		
Alfalfa mosaic virus	AMV	*Alfamovirus*				Y	
Beet curly top virus	BCTV	*Curtovirus*				N	Not mechanically transmitted
Beet ringspot virus	BRSV	*Nepovirus*				Y?	BRSV was formerly characterised as a strain of *Tomato black ring virus* (Harrison, 1958)
Belladona mottle virus	BeMV	*Tymovirus*				Y	
Cherry leaf roll virus	CLRV	*Nepovirus*				Y	
Colombian potato soil-borne virus	CPSbV	*Pomovirus*				Y	
Cucumber mosaic virus	CMV	*Cucumovirus*				Y	
Eggplant mottled dwarf virus	EMDV	*Nucleorhabdovirus*				Y	
Groundnut bud necrosis virus	GBNV	*Orthotospovirus*				Y	EPPO Standard in preparation
Groundnut ringspot virus	GRSV	*Orthotospovirus*				Y	
Impatiens necrotic spot virus	INSV	*Orthotospovirus*			A2	Y	
Papaya mosaic virus	PapMV	*Potexvirus*				Y	
Pepino mosaic virus	PepMV	*Potexvirus*	A2[†]			Y	PM 7/113 (EPPO, 2013a)
Potato aucuba mosaic virus	PAMV	*Potexvirus*				Y	
Potato virus B	PVB	*Nepovirus*				Y	Nepoviruses are mechanically transmitted but transmission of this virus needs to be determined (De Souza *et al.*, 2017)
Potato virus H	PVH	*Carlavirus*				Y	
Potato virus U	PVU	*Nepovirus*				Y	
Potato yellow blotch virus	PYBV	*Potyvirus*				Y	PVA polyclonal antibodies will probably detect PYBV (Nisbet *et al.*, 2019)
Potato yellow dwarf virus	PYDV	*Nucleorhabdovirus*			A1	Y	
Potato yellow mosaic virus	PYMV	*Begomovirus*				Y	Rare example of a begomovirus that seems to be reliably mechanically transmitted (Roberts *et al*., 1988)
Sowbane mosaic virus	SoMV	*Sobemovirus*				Y	
Tobacco mosaic virus	TMV	*Tobamovirus*				Y	
Tobacco necrosis virus D	TNV	*Betanecrovirus*				Y	
Tobacco streak virus	TSV	*Ilarvirus*				Y	
Tomato black ring virus	TBRV	*Nepovirus*				Y	
Tomato chlorosis virus	ToCV	*Crinivirus*				N	Not mechanically transmitted PM 7/118 EPPO (2013b)
Tomato chlorotic spot virus	TCSV	*Orthotospovirus*				Y	EPPO Standard in preparation
Tomato leaf curl New Delhi virus	ToLCNDV	*Begomovirus*				Y?	Example of a begomovirus that is mechanically transmitted but may be isolate dependent
Tomato mosaic virus	ToMV	*Tobamovirus*				Y	
Tomato mottle Taino virus	ToMoTV	*Begomovirus*	A1[¶]	IA1		N?	Transmission by biolistic inoculation of tomato and pepper plants (Ramos *et al.*, 1997) but reliability of mechanical inoculation needs establishing PM 7/50 (EPPO, 2005b)
Tomato ringspot virus	ToRSV	*Nepovirus*	A2	IA1	A2	Y	PM 7/49 (EPPO, 2005a)
Tomato severe rugose virus	ToSRV	*Begomovirus*				N	Poorly or not mechanically transmitted
Tomato yellow ring virus	TYRV	*Orthotospovirus*				Y	
Tomato yellow leaf curl virus	TYLCV	*Begomovirus*	A2[††]	IA2		N	Poorly mechanically transmitted PM 7/50 (EPPO, 2005b)
Tomato yellow vein streak virus	ToYVSV	*Begomovirus*				Y?	Mechanical transmission from *N. benthamiana* to *N. benthamiana* (Albuquerque *et al.*, 2010) but transmission from potato has not been tested
Wild potato mosaic virus	WPMV	*Potyvirus*				Y	

[†]Commission Decision of 27 February 2004 on measures to prevent the introduction into and the spread within the Community of Pepino mosaic virus.
[¶]EPPO lists ToMoTV and other Geminiviridae of capsicum and tomato.
[††]EPPO lists TYLCV and other related viruses.

probes): for RNA use *nad*5 and for DNA use COX or Eukaryotic 18S or 28S rRNA gene (fragment) as relevant (see Appendix 6). The inclusion of an internal control test is recommended to eliminate the possibility of PCR false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors. This will require the primer (and probe) concentrations to be optimised to prevent low pest levels being outcompeted by high levels of plant nucleic acids.

Confinement procedures

Stringent confinement procedures should be applied within quarantine since, if infected material is present, there is a risk of cross-infection of uninfected material that may escape detection. Confinement procedures should therefore be designed to prevent cross-infection but should include rules to allow holding and re-testing of material, or even destruction, if sufficient guarantees cannot be given that cross-infection has not occurred. All procedures should be documented (see below). Examples of confinement procedures are described elsewhere, but basic procedures include the following:

1. For material *in vitro*, actions in handling germplasm should be recorded chronologically, so that material can, if necessary, easily be checked for cross-infection if infected material is detected later. Stringent aseptic techniques and procedures should be applied, including autoclaving instruments or using a glass-bead sterilizer between units of each line (flame sterilization using methanol/ethanol may not always be effective but may be used within each unit) and cutting over a sterile disposable surface.
2. In the glasshouse, buffer zones should be established between the glasshouse entrance and the compartments where the plants to be tested are grown. Access should be restricted to persons directly involved in growing and testing of the plants, and staff should use protective clothing. Potential vectors should be monitored by regular inspection of plants and sticky traps. Screens (height up to 60 cm) for separation may be used to prevent potential contact/mechanical transmission between lines. During handling of plants, sterilized instruments and new disposable gloves should be used between each line. Glasshouse procedures are particularly important for quarantine based on *in vivo* procedures where plant material may not have received testing before being planted into the glasshouse and material is released from the glasshouse at the end of the quarantine procedure.

Administration of the procedure

The NPPO should be responsible for administration and monitoring of the procedure. The post-entry quarantine facilities, and all testing in post-entry quarantine, should be subject to its control or supervision. All procedures used in post-entry quarantine testing should be documented and records kept for at least 10 years of all tests done on the material and results, in a manner ensuring traceability. For potentially controversial positive results, isolates or material should be kept for at least 1 year after reporting the results, to allow for further or independent investigation if requested by the NPPO of the exporting country.

Material subject to the procedure should be imported only with an import permit. It is not the responsibility of the NPPO of the importing country to verify the provenance or identity of the material. Importers should be informed that all material is received on the understanding that the line or accession is as stated on the import permit.[8]

Material should be released from post-entry quarantine only if is derived from plants which have given negative results in the tests specified and has been inspected and found free from symptoms of disease during a complete vegetative cycle. For quarantine based on micropropagation, only microplants should be released. For procedures based on *in vivo* propagation, glasshouse-grown plants or tubers are released. The released material should be accompanied by a Germplasm Health Statement that specifies the tests done and the results.

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[8]There is also a small risk that imported vegetative material may not be true to phenotype and that mutations may arise during micropropagation. Importers should be made aware of this and advised to reduce the risk of producing solely aberrant material by importing more than one 'clone' of each line for quarantine testing.

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Appendix 1 – Example of propagation programmes based on *in vitro* culture for material received as microplants, tubers and true potato seeds

The propagation pathways should be designed to minimize the risk of failing to detect pests, particularly bacteria and some viruses, because of uneven distribution or low concentration.

In general:

- test each unit of candidate material (100% testing)
- adopt strict confinement procedures
- surface-sterilize tubers, seeds and sprouts using, for example, 2.5% sodium hypochlorite (sodium hypochlorite is equivalent to 8–10% active chlorine) for 10–20 min, followed by washing in sterile distilled water
- use a suitable medium for growth of microplants, e.g. Murashige and Skoog (M&S) medium without growth regulators (Sigma Cat. No M-5519) and with 30 g L^{-1} sucrose and 5–8 g L^{-1} Oxoid No.3 Agar (or equivalent)
- incubate microplants at 18–22°C, with 14–16 h daylight, under cool white fluorescent tubes.

Material received as vegetative material should be tested to ensure pest freedom using two independent tests with each test used at a different point in the propagation programme, e.g. for viruses, ELISA on microplants and bioassay on glasshouse plants.

For material received as true potato seeds the minimum requirement is that glasshouse-grown plants derived from true potato seeds are tested once for the pospiviroids and seed-transmissible virus species *Andean potato latent virus*, *Andean potato mild mosaic virus, Arracacha virus B*-oca strain, *Potato black ringspot virus, Potato virus T* and Potato yellowing virus using one of the tests described in Table 1. Testing microplants alone is not recommended since the reliability of testing microplants of *Solanum* spp. is unknown.

Material received as microplants

Request up to 5 microplants (derived from the same Mother Plant) for each line (1 microplant per tube). On receipt, inspect microplants closely for the absence of fungi, bacteria and arthropod pests, particularly mites and thrips. Destroy infested material, although material contaminated with saprophytic endogenous bacteria may be quarantine-tested at the discretion of the NPPO. Before subculturing, destructively test 1 microplant from each line for pospiviroids and 1–2 microplants for *C. m.* subsp. *sepedonicus* and the RSSC. These tests are optional, but testing at this stage reduces the risk of propagating infected material. Any of the test methods described may be used.

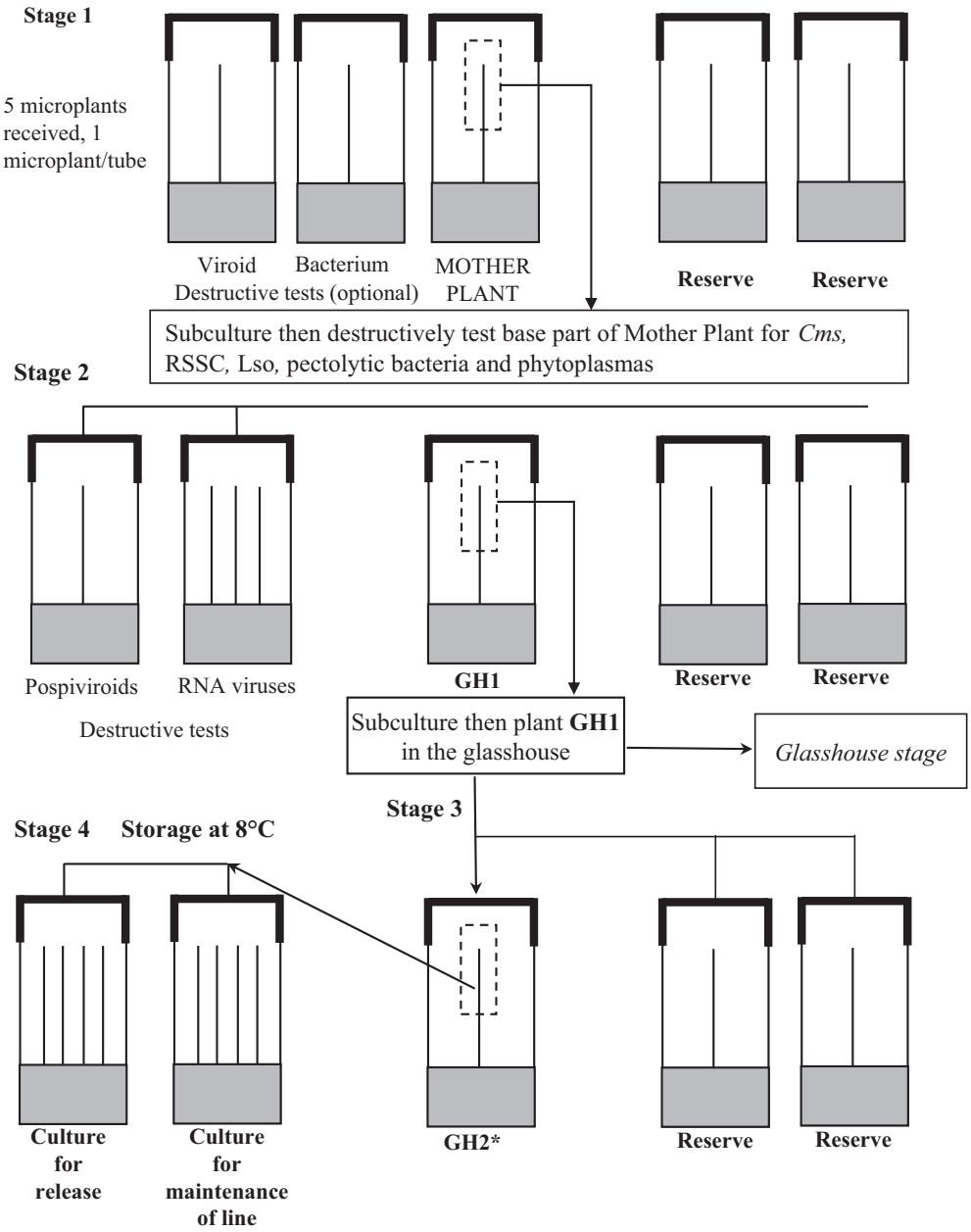
If the above tests are negative, select one of the remaining microplants (the Mother Plant, in Stage 1 in Figs 1 and 3) for full quarantine testing and subculture onto M&S medium (one or more microplants per tube depending on the intended use). Destructively test the stem base of the Mother Plant for *C. m.* subsp. *sepedonicus*, RSSC, '*Ca.* Liberibacter solanacearum', '*Ca.* Phytoplasma spp.' and pectolytic bacteria. Once the subcultures have grown, destructively test for pospiviroids and viruses (Stage 2 cultures in Figs 1 and 3). If test results are negative subculture GH1 to GH2 and after regrowth plant GH1 in the glasshouse. Subculture GH2 for storage and release following the propagation pathway shown in Figs 1 and 3. Destructively test Stage 2 cultures. GH2 should be planted in the glasshouse, for example, if GH1 fails to grow.

Grow GH1 in the glasshouse through a complete vegetative cycle. Inspect regularly for symptoms of disease and test for pospiviroids, viruses, '*Ca.* Liberibacter solanacearum' and '*Ca.* Phytoplasma spp.'. At the end of the growing cycle, harvest tubers, inspect and test for *C. m.* subsp. *sepedonicus* and the RSSC. Also test tubers for '*Ca.* Liberibacter solanacearum' and '*Ca.* Phytoplasma spp.' if glasshouse plants have not been tested. Cut a proportion of tubers and check for symptoms of disease, e.g. brown rot, ring rot, spraing and zebra chip.

Complete the record sheets. The person responsible for post-entry quarantine verifies by signature that the material has passed or failed the tests. If material passes the tests, maintain only microplants derived from GH2. Release stored microplants with a Germplasm Health Statement. Destroy all other microplants and the glasshouse material.

Material received as tubers

For each line, receive up to 5 tubers, surface-sterilize. Inspect for disease symptoms. If these are present only

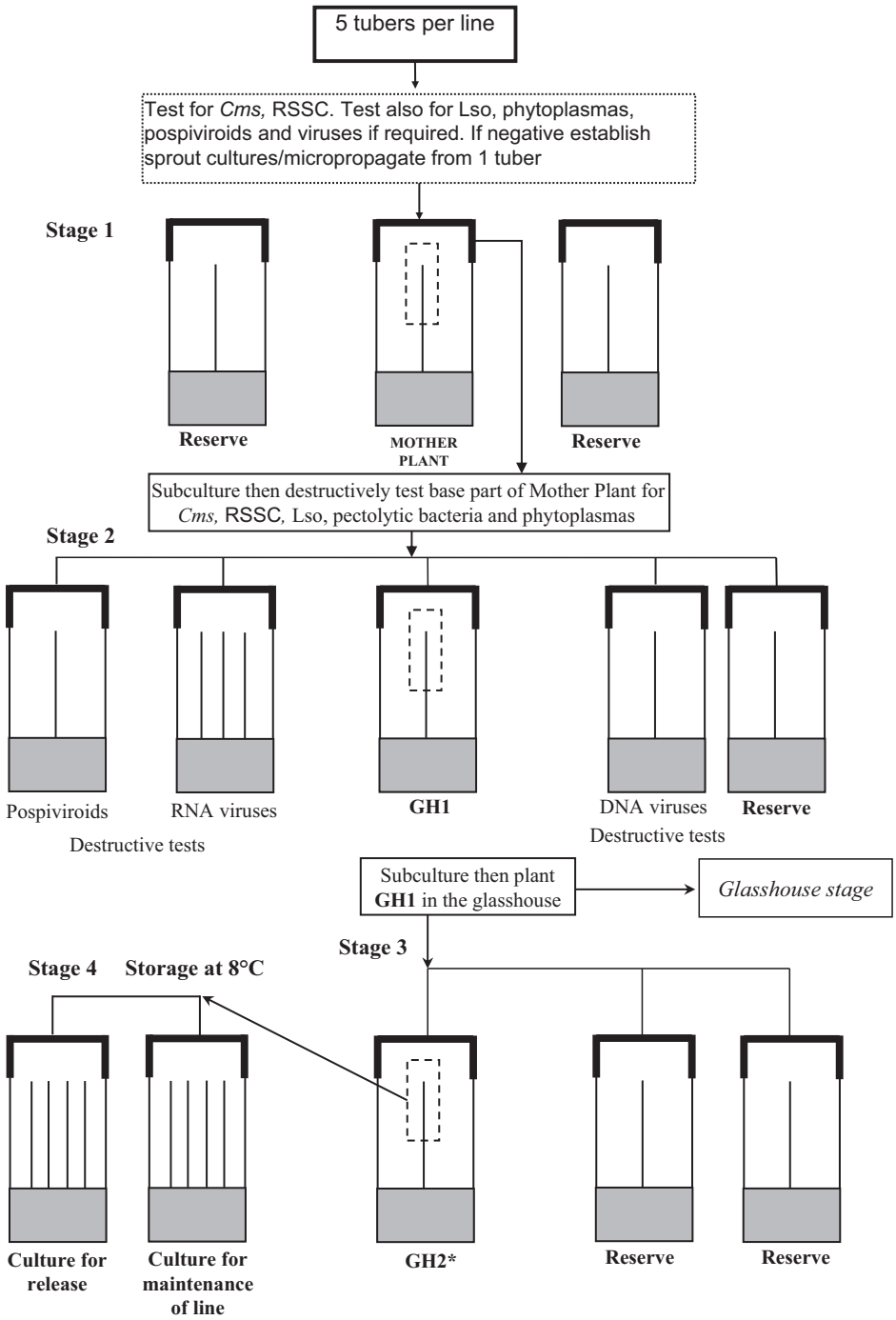


***GH2** for use as backup, if **GH1** dies or symptoms in **GH1** require further investigation.

Fig. 3 Detail of propagation pathway for material received as microplants (up to glasshouse stage).

continue at the discretion of the NPPO. Place the tubers in suitable containers and incubate in the dark at 15–20°C. Test each tuber for *C. m*. subsp. *sepedonicus* and the RSSC. Test tubers for pospiviroids, '*Ca*. Liberibacter solanacearum' and '*Ca*. Phytoplasma spp.' if required. The tests to use are described in 1. Once tubers have sprouted, sprouts from each tuber may be tested but although this test is optional it will prevent obviously infected material entering the propagation programme.

Once the tubers have sprouted, select one tuber from each line, remove the sprout(s), surface-sterilize them and excise at least 5 nodes from the sprout(s) and plant onto M&S Medium. Once the sprout node cultures have established, select the 'best' culture (the Mother Plant in Stage 1 of Fig. 3) and subculture it to produce plants for testing, planting in the glasshouse (GH1 and GH2) and for storage or release, following the propagation pathway shown in Figs 1 and 4. From this point on, the procedure is the same



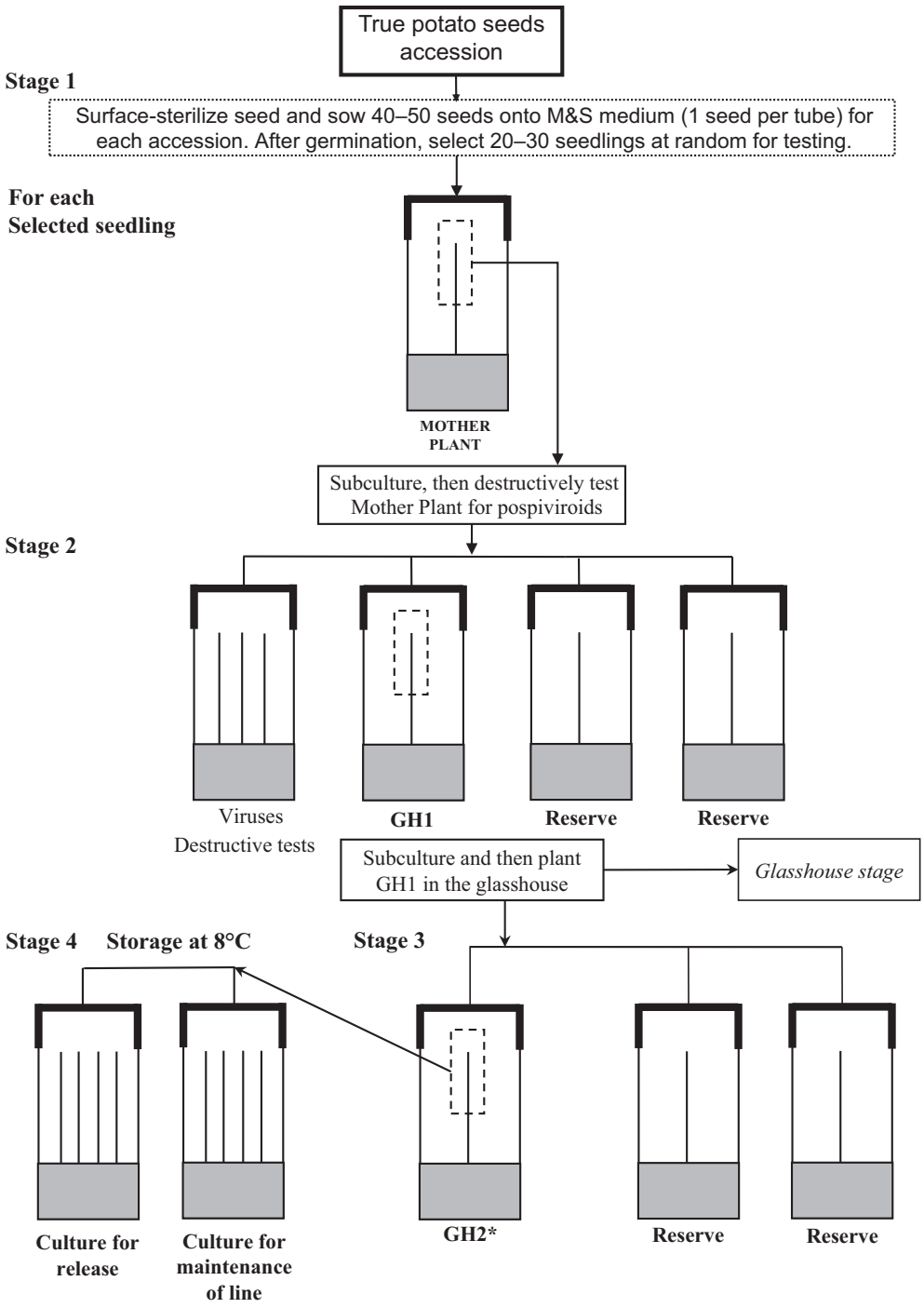
***GH2** for use as backup, if GH1 dies or symptoms in **GH 1** require further investigation.

Fig. 4 Example of propagation pathway for material received as tubers (up to glasshouse stage).

as for microplants, starting at 'Destructively test the stem base of the Mother Plant for *C. m.* subsp. *sepedonicus*…'

Material received as true potato seeds

On receipt, inspect true potato seeds closely for the absence of arthropod pests. Treat infested material by storage at –20°C for 7 days. For each accession, surface-sterilize 30–50 seeds. Aseptically transfer each surface-sterilized seed to a tube containing M&S medium. Select at least 20 seedlings (the Mother Plants) for full testing (note that only tested plants will be released). When sufficiently grown (about 5 cm) subculture the Mother Plant to produce microplants. Allow the remaining seedling-microplants to regrow and



***GH2** for use as backup, if **GH1** dies or symptoms in **GH1** require further investigation.

Fig. 5 Example of propagation pathway for material received as true potato seeds (up to glasshouse stage).

subculture these to produce plants for virus testing (if required), planting in the glasshouse, and for storage and release (see Figs 2 and 5). Allow the Mother Plant to regrow and test for pospiviroids. If required test the subcultures (Stage 2 cultures in Figs 2 and 5) for the seed-transmissible virus species *Andean potato latent virus*, *Andean potato mild mosaic virus, Arracacha virus B*-oca strain, *Potato black ringspot virus, Potato virus T* and Potato yellowing virus.

For the plant in the glasshouse allow it to grow through a complete vegetative cycle. Inspect regularly for symptoms of disease and test for pospiviroids and seed-transmissible

viruses if not tested previously. From this point on, the procedure is the same as for microplants, starting at 'If material passes the tests, maintain …'

Appendix 2 – Example of propagation programmes based on *in vivo* procedures for material received as microplants, tubers and true potato seeds

In general:

- Grow candidate material in the glasshouse in pest-free compost, test each unit (100% testing) and, if it passes the tests, release the plants or progeny tubers.
- Adopt strict confinement procedures.

For vegetative material, plants should be tested in the glasshouse for the pests in 1 using two independent tests which, preferably, are based on different biological principles, one of which should be a bioassay for viruses that are mechanically transmissible. For viruses which are not mechanically transmitted, or are not reliably mechanically transmitted, another independent test should be used.

Additional viruses have been reported to infect potato or related species naturally (Jeffries & Lacomme, 2018) and these are listed in Table 2. Although most of these may be detected using bioassay, an additional test based on a different biological principle may be used to reduce the uncertainty of detection, particularly if the plant material originates from a country where these viruses are known to occur. For viruses which are not mechanically transmitted, or not reliably mechanically transmitted, another independent test should be used.

If disease symptoms are present, but none of the tests is positive, then electron microscopy following EPPO Standard PM 7/126 *Electron microscopy in diagnosis of plant viruses* and/or high-throughput sequencing (also known as next-generation sequencing) should be considered (Boonham *et al*., 2014; Massart *et al*., 2014).

Material received as microplants

Request up to 5 microplants (derived from the same Mother Plant) for each line (1 microplant per tube). Use 1 of the microplants for further propagation (now called the Mother Plant) and establish 3 daughter plants. Keep the remaining microplants as a back-up. Plant the Mother Plant and 1 of the daughter plants and keep the 2 other daughter plants (sisters) for release. Plant both mother and daughter plants in separate pots (11 cm diameter or larger) in pest-free compost under humid conditions in the glasshouse. After about 10 days, lower the humidity to normal glasshouse conditions. For each plant, allow only one stem to grow. Remove all lateral shoots from the main stem. Alternatively, more stems may be allowed to grow, but it is important that all of these are then sampled for testing. If either the mother or daughter plant fails to establish, both plants are destroyed and the procedure starts again with another of the delivered microplants.

When the plants reach a length of about 25 cm (the stage at which first flowers are formed or will be formed soon) test for the viroids and viruses listed in Table 1 with additional tests for the viruses listed in Table 2 if required. After these have been done and some tubers have formed, test the stem of the plant for *C. m*. subsp. *sepedonicus* and the RSSC.

Complete the record sheets. The person responsible for post-entry quarantine verifies by signature that the material has passed or failed the tests. If both the Mother Plant and daughter plant pass the tests (and there are no concerns about cross-infection), release as appropriate the two plants, the newly formed tubers, or the two (sister) microplants which had been kept *in vitro*, with a Germplasm Health Statement.

Material received as tubers

For each line, import 2–5 tubers. One tuber or more, if requested by the customer, will be fully quarantine-tested. The remaining tubers will be partially processed and held in reserve should they be required. Dormancy may be broken by storing the tubers at 4°C for several weeks and then by raising temperatures to 18–20°C. Test each tuber for *C. m*. subsp. *sepedonicus* and the RSSC. Tubers may also be tested for '*Ca*. Liberibacter solanacearum' and '*Ca*. Phytoplasma spp.' if required. It may also be useful to conduct preliminary testing of tuber sap or sprouts for other pathogens in order to avoid unnecessary propagation of infected material.

From each tuber take one or more eye-plugs, soak in a solution of 2 µg mL^{-1} gibberellic acid for 15 min and then plant each eyeplug in a separate pot (11 cm diameter or larger).

When the eye-plug plants have reached a length of about 25 cm (the first flowers may be formed around this stage) test for the viroids and viruses listed in Table 1 on each plant, with additional tests for the viruses listed in Table 2 if required. After these tests have been completed, and some tubers have formed, test the stem of each plant for *C. m*. subsp. *sepedonicus*, the RSSC, '*Ca*. Liberibacter solanacearum' and '*Ca*. Phytoplasma spp.' if not done earlier.

If the plant passes the tests (and there are no concerns about cross-infection), release the eye-plug plants or the newly formed progeny tubers with a Germplasm Health Statement.

Material received as true potato seeds

For each accession, sow the seeds individually in a row in pest-free compost. When the seedlings have 2–4 leaves, transplant at least 20 seedlings individually into pots (e.g. 11 cm diameter or larger). When the seedling-plants have reached a length of about 25 cm (the first flowers may be formed around this stage) carry out the tests on each plant for PSTVd and the seed-transmissible virus species *Andean*

potato latent virus, *Andean potato mild mosaic virus, Arracacha virus B*-oca strain, *Potato black ringspot virus, Potato virus T* and Potato yellowing virus. Optionally, excess seedlings which are not grown on further may be tested for pospiviroids.

If material passes the tests (and there are no concerns about cross-infection), release the tested plants or tubers (bulking tubers for each accession) with a Germplasm Health Statement.

Appendix 3 – Examples of molecular tests for viroids and viruses when available

See Tables 3 and 4.

Appendix 4 – Virus bioassay

Prepare inoculum by grinding leaf tissue in phosphate inoculation buffer, e.g. 0.02 M phosphate buffer (Na_2HPO_4 2.5 g L^{-1}, NaH_2PO_4 0.8 g L^{-1}) pH 7.4 + 2% w/v polyvinylpyrrolidone (PVP, MW 10000). If another buffer is used, it should be tested for suitability against all viruses listed for testing. Use at least 2 plants from each indicator plant species. Inoculate young (3–6 leaf stage), soft, actively growing test plants by rubbing plant sap on leaves lightly dusted with carborundum (400–600 mesh). After inoculation, rinse the sap from the leaves with water and grow the plants at 18–25°C for at least 3–4 weeks under slight shade. The shorter growing time may be used only if the specific environmental conditions used in the glasshouse or growth room, or the species of indicator plant used, have been shown and have been documented as allowing reliable detection of the listed viruses within 3 weeks. Shading (e.g. by covering with paper) prior to and after inoculation may enhance susceptibility and symptom development. Inspect plants at least once a week for symptoms and record that the plants have been inspected and if symptoms are observed these should be recorded. Suitable indicator plants for each virus are indicated in Table 5.

The first version of this Standard listed, for vegetative material, *Chenopodium amaranticolor*, *C. murale, C. quinoa*, *Datura metel*, *Nicotiana clevelandii and N. tabacum* (or clone A 6) and for true potato seeds *C. amaranticolor*, *C. murale* and *C. quinoa,* although these may not reliably detect Potato yellowing virus (PYV) and some other mechanically transmitted viruses, e.g. *Potato latent virus* and *Potato virus P*. For true potato seeds, *N. occidentalis*-P1 and *N. hesperis*-67A can be used (Verhoeven & Roenhorst, 2000) and the three-indicator set *C. quinoa, N. occidentalis*-P1 and *N. hesperis*-67A has been proposed as sufficient to detect most of the viruses of concern in this Standard (Verhoeven & Roenhorst, 2003). Since bioassay may be influenced by a number of variables, including indicator plant accession (van Dijk & Cuperus, 1989) and environmental conditions, each laboratory should test each indicator plant against each virus of concern before use.

Appendix 5 – Molecular tests for bacteria

See Table 6.

Table 3. Examples of molecular viroid and virus tests for detection at the genus level

Genus	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′-3′	Author	International standard	Validation data*	Laboratory
Viroid genus								
Pospiviroid (CSVd, PSTVd, TPMVd)	Conventional RT-PCR	Pospi1-FW	Pospi1-RE		Verhoeven *et al.* (2004)	ISPM 27 DP 07		NL†, FR‡
	Conventional RT-PCR	Pospi2-FW	Pospi2-RE		Verhoeven *et al.* (2017)			NL†
	Real-time RT-PCR (Taqman)	Multiplex	Multiplex	Multiplex	Botermans *et al.* (2013)	ISPM 27 DP 07	NAK-NL for potato leaves PSTVd, TPMVd detected	NL‡
Virus genus								
Begomovirus (PYMV, ToLCNDV, ToMoTV, ToSRV, TYLCV, ToYVSV)	Conventional PCR	AV494	AC1048		Wyatt & Brown (1996) (for detection of ToLCNDV a reduced annealing temperature of 52°C must be used		Tests subject to validation in Euphresco project	UK‡,§, NL‡, IT¶
		SPG1	SPG2		Li *et al.* (2004)			MPI‡
		DengA	DengB		Deng *et al.* (1994)			NL¶, IT¶
Carlavirus (PotLV, PVH, PVM, PVS)	Conventional RT-PCR	Car-F2b	Not1pdt		Nie *et al.* (2008)			UK‡,§
		RepF3	RepR1		Unpublished**		NPPO-NL, PotLV, PVH, PVM, PVS detected in potato	NL¶
Comovirus (APMoV)	Conventional RT-PCR	Como1F	Como1R		Perez-Egusquiza *et al.* (2014)		APMoV detected in indicator plants	PE¶, NZ‡
Crinivirus (PYVV, ToCV)	Conventional RT-PCR	CriniRdRp251F	CriniRdRp 995R††		Wintermantel & Hladky (2010)		ToCV detected	
Curtovirus (BCTV)	Conventional RT-PCR	BCTV2F	BCTV2R		Strausbaugh *et al.* (2008)		BCTV detected in potato	NZ‡, UK‡,§
Ilarvirus (TSV, PYV)	Conventional RT-PCR	Ilar2F5	Ilar2R9		Untiveros *et al.* (2010)		PYV detected in potato	NZ‡
Nucleorhabdovirus (PYDV, EMDV)	Conventional RT-PCR	RhabF††	RhabR††		Lamprecht *et al.* (2009)		Not tested for these species	
Nepovirus (subgroup A) PBRSV	Conventional RT- PCR	NepoA-F	NepoA-R		Wei & Clover (2008) (2 step RT-PCR)	EPPO PM 7/002 (Wei & Clover test adapted to 1 step RT-PCR)		
Nepovirus (subgroup B) BRSV, PVB, TBRV	Conventional RT- PCR	NepoB-F	NepoB-R		Wei & Clover (2008) (2 step RT-PCR)		Not tested against PVB	UK‡
Nepovirus (subgroup C) CLRV, PVU, ToRSV	Conventional RT-PCR	Nepo-C(s)	Nepo-C(a)		Digiaro *et al.* (2007) (2 step RT-PCR)		Not tested against CLRV and PVU	
Orthotospovirus (GBNV, GRSV, INSV, TCSV, TSWV, TYRV)	Conventional RT-PCR	gM410-F	gM410-F		Chen *et al.* (2012)	ISPM 27 DP 24	LNPVAAL TCSV, TYRV, TSWV detected (not potato)	
	Conventional RT-PCR	Multiplex	Multiplex		Hassani-Mehraban *et al.* (2016)	EPPO PM7 in preparation		NL†

(continued)

Table 3 *(continued)*

Genus	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′-3′	Author	International standard	Validation data*	Laboratory
Pomovirus (CPSbV, PMTV)	Conventional RT-PCR	††	††		††			
Potexvirus (PAMV, PapMV, PepMV, PVX)	Conventional RT-PCR	Potex-5	Potex-1RC		van der Vlugt & Berendsen (2002)		PAMV, PVX detected	NL¶, UK‡,§
Potyvirus (PVA, PVV, PVY, PYBV, WPMV)	Conventional RT-PCR	PV2	POT1		Gibbs & Mackenzie (1997) (for POT2) and Colinet *et al.* (1994) (for POT1)		PVA, PVV, PVY detected	UK‡,§
		Oligo 1n	Oligo 2n		Marie-Jeanne *et al.* (2000)			MPI‡
Tobamovirus (TMV, ToMV)	Conventional RT-PCR	TobamodF	TobamodR		Li *et al.* (2018)			
Tobravirus (TRV)	Conventional RT-PCR‡‡	H42	H43		Unknown			NL¶
Tymovirus (APLV, APMMV, BeMV)	Conventional RT-PCR	EM13	EM14		Kreuze *et al.* (2013)	EPPO PM7/132	Test under evaluation in a Euphresco project	NL¶

*It is recommended to consult http://dc.eppo.int/validationlist.php as additional information may be available there.

†Test used in potato quarantine testing (secondary test) in this laboratory.

‡Test used in potato quarantine testing (primary test) in this laboratory.

§Test ISO 17025 accredited for testing potato in this laboratory.

¶Test available but may not be currently used for potato quarantine testing in this laboratory.

**Primers: RepF3 (5′-TGC ACN GAR TCN GAY TAY GAR GC-3′) and RepR1 (5′-CAC ATR TCR TCN CCN GCR AAV CA-3′), described by Pham *et al.* (2007).

††Test currently not available.

‡‡Primers H42 (5′-TGT TTG AGA TTG GCG TTT GGC C-3′) and H43 (5′-GGG CGT AAT AAC GCT TAC GTA G-3′).

Table 4. Examples of more specific viroid and virus tests

Species/genus	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′–3′	Author	International standard	Validation data*	Laboratory
Viroid species								
Chrysanthemum stunt viroid /Pospiviroid	Conventional RT-PCR	Vir 1	Vir 2		Mumford *et al.* (2000b)	EPPO PM 7/006 (1)	Cross reacts with some other pospiviroids	
	Real-time PCR (Taqman)	CSVd 220F	CSVd 297R	CSVd 249T		EPPO PM7 on pospiviroids (in preparation)	Specific for CSVd	
Potato spindle tuber viroid/Pospiviroid	Conventional RT-PCR	PSTVd69s	PSTVd89c		Weidemann & Buchta (1998)		Tested on potato	DE†
		3H1-F	2H1-R		Shamloul *et al.* (1997)	ISPM 27 DP 07	Cross-reacts with some other pospiviroids	NL‡
		Pospi2-FW	Pospi2-RE		Verhoeven *et al.* (2017)			NL‡
	Real-time PCR (Taqman)	PSTV-231F	PSTV-296R	PSTV-251T	Boonham *et al.* (2004)		NAK PSTVd detected in potato leavesCross-reacts with some other pospiviroids	DE§, NL‡ FR§
Virus species								
Alfalfa mosaic virus/ Alfamovirus	Conventional RT-PCR	AM-F or AM-F2	AM-R or AM-R2		Xu & Nie (2006)		Tested on potato	
		P4	P3		Navarre *et al.* (2009)			
Andean potato latent virus/Tymovirus	Conventional RT-PCR	AP5	EM10		Kreuze *et al.* (2013)		Tested on potato	NZ§
Andean potato mild mosaic virus/ Tymovirus								
Arracacha virus B-oca strain/*Cheravirus*	Real-time RT-PCR (Taqman)	AVB-5388F	AVB -5611R	AVB5506-31P	Tang (2016)			NZ§
Colombian potato soil-borne virus	Conventional RT-PCR	CDF1	CDF2		Gil *et al.* (2016)		Tested on potato	
Potato leafroll virus/ Polerovirus	Real-time RT-PCR (Taqman)	PLRV-FOR	PLRV-P	PLRV-REV	Boonham *et al.* (2009)		Tested on potato	UK§,¶
		PLRV-1 For	PLRV-1 Rev	PLRV-1 probe	Agindotan *et al.* (2007)		Tested on potato	FR§
Potato mop-top virus/ Pomovirus	Real-time RT-PCR (Taqman)	PMTV-1948F	PMTV-2017R	PMTV-1970	Mumford *et al.* (2000a)		Tested on potato	NZ§, UK§, FR§
Potato virus X	Real-time RT-PCR (Taqman)	PVX-1 For	PVX-1 Rev	PVX-1 probe	Agindotan *et al.* (2007)		Tested on potato	FR§
Potato virus T/ Tepovirus	Conventional RT-PCR	PVT-1	PVT-2		Lizárraga *et al.* (2000)		Tested on potato	PE‡
Potato yellow vein virus/Crinivirus	Conventional RT-PCR	PYVV 414 F	PYVV 670R		López *et al.* (2006)		Tested on potato	FR§
		PYVVCPF	PYVVCPR		Offei *et al.* (2004)		Tested on potato	PE†
		PYVV 591F	PYVV 670R	PYVV 615T	López *et al.* (2006)		Tested on potato	

(continued)

Table 4 *(continued)*

Species/genus	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′–3′	Author	International standard	Validation data*	Laboratory
	Real-time RT-PCR (Taqman)							PE‡, NL§, NZ§, UK§,¶
Potato yellowing virus/ Ilarvirus	Conventional RT-PCR	PYV-Rd-4F	PYV-Rd-4R		Monger (2012) Unpublished**		Tested on potato	UK§,¶
Tobacco necrosis virus D/Betanecrovirus	Conventional RT-PCR	FD	RD		Cardoso *et al.* (2004)		Tested on potato	UK§
Tobacco rattle virus/ Tobravirus	Conventional RT-PCR	A	B		Robinson (1992)		Tested on potato	
	Real-time RT-PCR (Taqman)	TRV 1466 F	TRV 1553 R	TRV 1489P	Mumford *et al.* (2000a)		Tested on potato	NL‡, NZ‡, UK§,¶ FR§
Tomato chlorosis virus/Crinivirus	Real-time RT-PCR (Taqman	ToCV 258F	ToCV 331R	ToCV probe	Morris *et al.* (2006)	EPPO PM 7/118	Tested on potato	UK§,¶, FR§
	Real-time RT-PCR (Taqman)	Toc-CoatD-Fw	Toc-CoatD-Rv	Probe-Toc-CP	Van der Vlugt *et al.* (2011)		Not tested on potato	NL‡
Tomato leaf curl New Delhi virus/ Begomovirus	Conventional PCR	ToLCNDV-1144F	ToLCNDV-2080R		Saison (2015) Unpublished††		Tested on potato	FR§
Tomato ringspot virus/ Nepovirus	Real-time RT-PCR (Taqman)	ToRSV-UTRf	ToRSV-UTRr	ToRSV-UTRp	Tang *et al.* (2014)		Tested on potato	FR§
Tomato spotted wilt virus/ Orthotospovirus	Real-time RT-PCR (Taqman)	TSWV-1 For	TSWV-1 rev	TSWV-1 probe	Mortimer-Jones *et al.* (2009)		Tested on potato	FR§

*It is recommended to consult http://dc.eppo.int/validationlist.php as additional information may be available there.

†Test used in potato quarantine testing (secondary test) in this laboratory.

‡Test available but may not be currently used for potato quarantine testing in this laboratory.

§Test used in potato quarantine testing (primary test) in this laboratory.

¶Test ISO 17025 accredited for testing potato in this laboratory.

**PYV-Rd-4F: CCA TGA AGT GCA (ag)RAG AAA AA-3′; PYV-Rd-4R: 5′-AAT CCA ACT (agt)GT GTA CCA GG (SASA, UK).

††ToLCNDV-1144F : 5′-CGCAGGTTGTGGTTGAAYTG-3′; ToLCNDV-2080R : 5′-GCYCGTGGWGGTCAACARAC-3′ (ANSES, FR).

‡‡Test currently not available.

Table 5. Indicator plant reactions against potato infecting viruses[*] and additional reactions from publications,[†] e.g. Jeffries (1998)

Virus	*Chenopodium amaranticolor*	*Chenopodium murale*	*Chenopodium quinoa*	*Nicotiana benthamiana*	*Nicotiana bigelovii*	*Nicotiana clevelandii*	*Nicotiana debneyi*	*Nicotiana hesperis –67A*	*Nicotiana occidentalis-P1*	*Nicotiana tabacum 'White Burley'*	Virus
AMV	+/+	nt	(+)/+	(+)/(+)	−/+	nt	−/+	+/+	+/+	(+)/+	AMV
APLV	−/(+) LS	L	−/(+) L!S	(+)/+	(+)/(+)c	−/(+)Sc	−/−S	+/+	+/+LcSc	−/−	APLV
APMMV	−/− S	nt	−/−	−/(+) L	(+)/(+)	nt	−/−	+/+	+/+	−/−	APMMV
APMoV	−/−	nt	−/−	−/+	−/+c	(+)/(+)Sc	(+)/−	+/+	+/+	(+)/−	APMoV
AVB-O	−/− LcSc	−/(+) c	(+)/−	−/−	−/(+)	nt	−/−	+/+	+/+ nsc	−/−	AVB-O
BCTV[‡]											BCTV[‡]
BeMV	nt	nt	L	nt	nt	nt	nt	nt	nt	LS	BeMV
BRSV	nt	nt	(+)/−	−/−	nt	nt	nt	nt	+/+	−/−	BRSV
CLRV	LS	nt	LS	nt	nt	nt	nt	nt	nt	LS	CLRV
CMV	+/−	nt	(+)/−	(+)/(+)	−/−	nt	−/−	−/+	(+)/+ L!	(+)/(+)	CMV
CPSbV	nt	nt	nt	S	nt	nt	nt	nt	nt	nt	CPSbV
EMDV[3]	nt	nt	nt	LS	nt	LS	LS	nt	nt	LS	EMDV[3]
GBNV	nt	nt	−/−	(+)/+	nt	nt	nt	nt	(+)/+	−/+	GBNV
GRSV	L	nt	(+)/−	(+)/+	nt	nt	nt	nt	+/+	+/+	GRSV
INSV	+/−	nt	(+)/−	(+)/(+)	−/−	nt	−/−	+/(+)S!	+/(+)	(+)/(+)	INSV
PapMV	L	nt	nt	nt	nt	nt	nt	nt	nt	nt	PapMV
PepMV	nt	nt	−/−	(+)/+	nt	nt	nt	nt	+/(+)	−/−	PepMV
PAMV	−/−	nt	−/−	−/−	−/−	nt	−/−S	+/+	+/+	−/−	PAMV
PBRSV	+/+	nt	+/+LcSc	+/+	+/−	nt	−/−	+/+	+/+c	−/− L!cSc	PBRSV
PLRV[‡]											PLRV[‡]
PotLV	−/−	L!S	−/−	−/−	−/− c	nt	−/− Sc	−/(+)	−/(+)Sc	−/−	PotLV
PMTV	−/− L	nt	−/− L	−/(+)	−/−	nt	−/− S!	(+)/(+)	−/(+)	−/−	PMTV
PVA	−/−	nt	−/−	−/− Sc	−/−	nsc	−/− Sc	−/+	−/+c	−/−S	PVA
PVB	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	PVB
PVH	ns	nt	+/− ns	−/+	nt	nt	nt	nt	+/+	nt	PVH[§]
PVM	−/−	ntL	(+)/−	−/−	−/−	Sn	−/− Ln	(+)/+	+/+LcSc	−/−	PVM
PVP	−/− L	nt	−/−	−/−	−/−	nt	−/−S	−/−	−/+c	−/−	PVP
PVS	−/(+) L	ntL	(+)/(+)LcSc	−/−	−/−	nt	−/− c	−/−	−/+c	−/−	PVS
PVT	−/+ L!Sc	S	−/+ L!Sc	−/−S	−/−	nt	−/−S!	−/+	−/(+)Sc	−/−	PVT
PVU	LS	nt	LS	nt	nt	nt	nt	nt	nt	SR	PVU
PVV	(+)/−	nt	−/−	−/+	−/−	S	−/−LcSc	+/+	+/+c	−/−Sc	PVV
PVX	+/−	L	+/−	(+)/(+)Sc	(+)/(+)	S	(+)/(+)S	+/(+)	+/+c	(+)/(+)LcSc	PVX
PVY	(+)/(+)	L	(+)/(+)	−/+c	−/(+)	nt S	−/(+)S	(+)/+	(+)/+LcSc	(+)/(+)LcSc	PVY
PYBV	ni	ns	ns	Sc	ns	ns	ns	Sc	LSc	ns	PYBV
PYDV	nt	nt	nt	nt	nt	LS	nt	nt	L!S	nt	PYDV
PYMV	nt	nt	nt	LS	nt	nt	nt	nt	nt	nt	PYMV
PYVV[‡]											PYVV[‡]
PYV	−/− L	ntL	L −/−	−/−	−/−	nt	−/− L	−/(+)	−/(+)	−/− L	PYV
SoMV	LS	LS	LS	nt	nt	nt	nt	nt	nt	nt	SoMV

(continued)

Table 5 *(continued)*

Virus	*Chenopodium amaranticolor*	*Chenopodium murale*	*Chenopodium quinoa*	*Nicotiana benthamiana*	*Nicotiana bigelovii*	*Nicotiana clevelandii*	*Nicotiana debneyi*	*Nicotiana hesperis –67A*	*Nicotiana occidentalis-P1*	*Nicotiana tabacum 'White Burley'*	Virus
TMV	+/−	nt	+/−	−/+	−/+	nt	−/+	−/+	(+)/+	−/+	TMV
TNV-D	+/−	nt	+/−	+/(+)	+/−	L	−/−	+/−	+/−	(+)/−	TNV-D
TRV	+/−	nt	(+)/(+)	−/(+)	+/(+)	L!S!	(+)/(+)	+/+S!	(+)/(+) L	(+)/(+)	TRV
TSV	+/−	nt	(+)/(+)	−/(+) S	−/−	nt	−/−	+/+	+/+	(+)/(+)	TSV
TBRV	+/+	nt	+/+ LcSc	−/(+)	−/+	LS	+/−	(+)/−L!cSc	+/+LcSc	(+)/+ LcSc	TBRV
ToCV[‡]											ToCV[‡]
TCSV	ntL	nt	+/−	+/+	nt	nt	S	nt	+/+	+/+	TCSV
ToLCNDV	nt	nt	−/−	−/(+)	nt	nt	nt	nt	−/(+)	−/−	ToLCNDV
ToMV	+/−S!	LS	+/(+)	(+)/+	−/+	nt	+/−	+/+	(+)/+	+/−	ToMV
ToMoTV[§]	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	ToMoTV[§]
ToSRV[‡]											ToSRV
TSWV	(+)/−	nt	(+)/−LS	(+)/(+)c	(+)/+	nt	(+)/(+)	(+)/+	(+)/(+)LcSc	(+)/(+)LcSc	TSWV
TYRV	L	nt	L	+/+	nt	nt	nt	nt	+/+	L!S!	TYRV
TYLCV[‡]											TYLCV[‡]
ToYVSV[§]	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	ToYVSV[§]
WPMV	−/−	nt	−/−	−/+	(+)/+	S	(+)/−	(+)/+	−/+	(+)/(+)	WPMV

Sometimes test plant reactions obtained by NPPO-NL, NPPO-UK and those from publications may differ. Reactions may depend on many factors, e.g. virus isolate, test-plant accession and climatic conditions such as temperature and light (season). Users are therefore recommended to test that the viruses can be detected under local conditions.

*Test plant reactions obtained by NPPO-NL: nt, not tested; symptoms local/systemic: +, symptoms for all isolates tested; (+), symptoms for only some of the isolates tested, −, no symptoms.

[†]Additional reactions from publications (e.g. Jeffries, 1998). L, local symptoms; S, systemic symptoms; R, symptoms may be followed by recovery so that no symptoms are seen; !, infection or symptoms may be absent and may depend on virus isolate; ni, not infected; ns, no symptoms but confirmed infected using ELISA; c, symptoms confirmed by NPPO-UK (Scotland).

[‡]Virus not mechanically transmitted or poorly transmitted.

[§]Reported to be mechanically transmitted but evaluation of indicator plants required before recommendations can be made.

Table 6. Examples of molecular tests for bacteria

Genus	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′–3′	Author	International standard	Validation data*	Laboratory
Clavibacter michiganensis subsp. *sepedonicus*	Conventional PCR	PSA-1	PSA-R		Pastrik (2000)	Council Directive 93/85/EEC as amended by Commission Directive 2006/56/EC		DE†, FR‡, NL‡, NZ‡
	Conventional PCR				Mills *et al.* (1997)	Council Directive 93/85/EEC as amended by Commission Directive 2006/56/EC PM7/042		F†
	Real-time PCR (TaqMan)	Cms 50-2F	Cms 50-133R	Cms 50-53T	Schaad *et al.* (1999)		Vreeburg *et al.* (2016) and van Vaerenbergh *et al.* (2017)	DE‡, NL‡
Ralstonia solanacearum species complex (of concern for potato these are *R. solanacearum* and *R. syzygii* subsp. *indonesiensis*)	Conventional PCR	OLI-1	Y-2		Seal *et al.* (1993)	Council Directive 98/57EC as amended by Commission Directive 2006/63/CE	Detects *R solancearum*, *R. pseudosolanacearum, R. syzygii* (all phylotypes)	FR†, NL†
	Conventional PCR	Ps-1	Ps-2		Pastrik & Maiss (2000)		Detects *R. solanacearum*, *R. pseudosolanacearum*	DE†
	Conventional PCR	RS-1-F	RS-1-R		Pastrik *et al.* (2002)	Council Directive 98/57EC as amended by Commission Directive 2006/63/CE PM7/021	Detects *R. solanacearum* (Phylotype II)	DE†,FR‡
	Real-time PCR	B2-I-F	B2-II-R	B2-P	Weller *et al.* (2000)	PM7/021	Detects *R. solanacearum* (Phylotypes IIB-1, IIB-2 and some other Phylotype IIB isolates of undetermined sequevar)	DE‡, NL‡
		RSSC-I-F	Rs-II-R	Rs-P	Weller *et al.* (2000)	PM7/021	Detects *R. solanacearum*, *P. pseudosolanacearum R. syzygii* (all phylotypes)	DE‡, NL‡
		Rs-I-F	Rs-II-R	RsP-55T	Vreeburg *et al.* (2016)		Detects *R. solanacearum*. No information on other species detected	DE‡, NL‡

(continued)

Table 6 *(continued)*

Genus	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′–3′	Author	International standard	Validation data*	Laboratory
'*Candidatus* Liberibacter solanacearum' (all haplotypes)	Conventional PCR	Lso TX 16/23F	Lso TX 16/23R		Ravindran *et al.* (2011)	ISPM 27 DP 21	Tested on potato	NL‡
	Real-time PCR (Taqman)	LsoF	HLBr	HLBp	Li *et al.* (2009)	ISPM 27 DP 21	Tested on potato	FR‡, UK‡
	Real-time PCR (Taqman)	CaLsppF	CaLsppR	CaLsolP	Teresani *et al.* (2014)	EPPO PM7/In preparation		
'*Ca.* Phytoplasma solani' (Stolbur); '*Ca.* Phytoplasma americanum' (Potato purple-top wilt)	Nested-PCR	P1/P7	R16F2n/ R16R2		P1 (Deng & Hiruki, 1991) and P7 (Schneider *et al.*, 1995)/ R16F2n (Gundersen & Lee, 1996) & R16R2 (Lee *et al.*, 1993)	ISPM 27 DP12		NZ‡
	Conventional PCR	NPA2F	NPA2R		Heinrich *et al.* (2001)			NL†
	Conventional PCR	STOL11f2	STOL11r1		Daire *et al.* (1997)			NL†
	Real-time PCR (Taqman)	UPH-F	UPH-R	UPH-PMGB	Malandraki *et al.* (2015)			NZ‡
	Real-time PCR (Taqman)	16S-333F	16S-396R	16S-370 LNA probe	Palmano *et al.* (2015)			UK§
	Real-time PCR (TaqMan)	JH-F1, JH-F-all	JH-R	JH-P-uni	Hodgetts *et al.* (2009)	ISPM 27 DP12 EPPO PM7/133		NL
	Real-time PCR (TaqMan)	16S rDNA F	16S rDNA r	16S rDNA	Christensen	ISPM 27 DP12		FR‡
Pectolytic bacteria (*Dickeya* spp and *Pectobacterium* spp.	Conventional PCR	Multiplex	Multiplex		Pritchard *et al.* (2013)			DE‡
	Conventional PCR	recAF	recAR		Waleron *et al.* (2002)			NZ‡

*It is recommended to consult http://dc.eppo.int/validationlist.php as additional information may be available there.
†Test used in potato quarantine testing (secondary test) in this laboratory.
‡Test used in potato quarantine testing (primary test) in this laboratory.
§Test available but may not be currently used for potato quarantine testing in this laboratory.
¶Test ISO 17025 accredited for testing potato in this laboratory.
**Test currently not available.

Appendix 6 – Examples of internal endogenous controls

Table 7. Examples of internal endogenous controls

Target	Method	Forward primer 5′–3′	Reverse primer 5′–3′	Probe 5′–3′	Author	International standard
Nad5 (mRNA of the mitochondrial NADH dehydrogenase subunit 5) gene	Conventional RT-PCR	*nad5*-s	*nad5*-as	-	Menzel *et al*. (2002)	ISPM 27 DP 07
	Real-time RT-PCR (Taqman)	*nad5*-F	*nad5*-R	*nad5*-P	Menzel *et al*. (2002) Botermans *et al*. (2013)	ISPM 27 DP 07
Cytochrome oxidase (DNA, mRNA and RNA)	Conventional PCR	COX1	COX2	-	Unpublished* *Product size 378 bp*	
	Real-time (RT-) PCR	COX-F	COX-R	COX-P	Mumford *et al*. (2004)	ISPM 27 DP 07
Eukaryotic 18S rRNA gene	Conventional PCR	NS-7-F	NS-8-R		Pastrik *et al*. (2002)	EU Directive 2006/56/EC
Eukaryotic 28S rRNA gene	Conventional PCR	28Sf	28Sr		Werren *et al*. (1995)	ISPM 27 DP 12

**COX1* CCG GCG ATG ATA GGT GGA; *COX2* GCC AGT ACC GGA AGT GA (SASA, UK).