DOI: 10.1111/epp.12950

PM 4/28 (2) Certification scheme for seed potatoes

SPECIFIC SCOPE

This Standard describes the production of certified seed potatoes. It is intended to be used by National Plant Protection Organizations (NPPOs) and official organizations in charge of certification, later called 'official organizations', in their capacity as bodies responsible for the design of systems for the production of healthy seed potatoes, for the inspection of such potatoes proposed for certification and for the issue of certificates (which may be a label).

The recommendations presented in this scheme are considered to be the minimum requirements for the production of seed potatoes. However, national authorities may decide to set stricter requirements in their national certification schemes based on the EPPO scheme, in order to take into account different conditions in their territories because of the prevalence of certain pests. This Standard does not apply to phytosanitary certification for export (see Standard PM 3/70; EPPO, 2006, under revision).

SPECIFIC APPROVAL AND AMENDMENT

First approved in 1999–09.
Revision approved in 2023–09.
Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

EPPO Schemes for the Production of Healthy Plants for Planting give details on the production of Nuclear Stock, and its multiplication through a number of stages such as Propagation Stock I, II, III and IV to meet the specified health standards (EPPO, 2009). To ensure that the EPPO Certification Scheme for Seed Potatoes complements and is compatible with the international UNECE *Standard S-1 concerning the marketing and commercial quality control of seed potatoes* (UNECE, 2021) the propagation stages used by the UNECE (Pre-basic, Basic and Certified[1]) are used in preference to the Propagation Stock stages normally used by EPPO.

The EPPO Certification Scheme for Seed Potatoes presents requirements for the production of certified seed potatoes to a quality standard, while accepting that a number of pests may be quarantine pests for certain countries (i.e. subject to national regulations, which have the objectives of containing or eradicating the pest). Consequently, seed potatoes produced in a country for domestic use or for export may have to satisfy additional requirements for these pests. As such the EPPO Certification Scheme for Seed Potatoes cannot include all these requirements, since they may differ according to the country. However, the scheme draws attention to the probable existence of such requirements; in particular, it refers to the requirements for seed potatoes moved within the EU, as laid down in the EU Marketing Directives (EU, 2020a, 2020b) and Plant Health Regulation (EU, 2022e) and to specific EU Implementing Regulations for the pests *Clavibacter sepedonicus* (EU, 2022c), *Globodera* spp. (EU, 2022a), *Ralstonia solanacearum* (EU, 2022b) and *Synchytrium endobioticum* (EU, 2022d).

Since most potato certification schemes in the EPPO region use nuclear stock produced in vitro (i.e. microplants), this is the main propagation method for nuclear stock described in this Standard. However, clonal selection is also a possibility for the production of nuclear stocks and this is described in Appendix 1.

2 | SPECIFIC DEFINITIONS

Seed potatoes

Tubers and microplants of cultivated tuber-forming *Solanum* spp., which are produced under an official certification system to meet specified requirements.

[1]The terms Pre-basic, Basic and Certified material have a different meaning in EPPO definitions (EPPO, 2009) than in UNECE: when these terms are used in EPPO PM 4 Standards, they only apply to material 'certified for sale'.

Official (in the context of PM4 Standards)

Established, authorized or performed by a national plant protection organization[2] (ISPM 5, IPPC, 2021) or by another organization designated by a government to administer potato seed certification.

Microplants of potato

Plants in vitro (including microtubers) of tuber-forming *Solanum* spp. [PM 3/62 *Production of pathogen-free microplants of potato* (EPPO, 2019a)]. Microplants could be used to produce plantlets or minitubers in a pest-free growing medium in a facility under specified protected conditions. This is further addressed in Section 3.4.1.1.

Minitubers of potato

Tubers produced from potato microplants in a pest-free growing medium in a facility under specified protected conditions [PM 3/63 *Production of pathogen-free minitubers of potato*; similar to ISPM 33 (IPPC, 2019)].

The terms 'Pre-basic' ('Pre-basic tissue culture (TC) class', 'Pre-basic seed class'), 'Basic' and 'Certified' are used mostly as defined in UNECE (2021). Other definitions are used as provided in the general introduction to the Series PM 4 (EPPO, 2009), such as 'candidate nuclear stock', 'certification scheme', and 'nuclear stock'. Other terms are used as defined in ISPM 5 (IPPC, 2021), such as 'field', 'free from', 'inspection', 'lot', 'pest', 'plants', 'quarantine pest', 'regulated non-quarantine pest' (RNQP), 'test'. When not already defined, other terms are used as defined in UNECE (2021). This is the case for e.g. 'chilling injury', 'external defect', 'field generation number', and 'rot'.

3 | OUTLINE OF THE SCHEME

The scheme has the aim of providing seed potatoes, which are free from certain pests and meet specified tolerances for others, and whose health status is attested by an official certificate (e.g. label). It does not cover farm-saved tubers or potato germplasm (tubers or microplants to be used as breeding material or true potato seeds). For the production of seed potatoes in the EPPO certification scheme, the following steps should be officially monitored.

1. Selection of individual candidate nuclear stock plants (tubers or microplants) of each cultivar, which have been derived from plants (mother plants) visually inspected for quality and trueness to type. Optional testing of the mother plants for pest freedom and cultivar identity.
2. Establishment of microplants from sprouts of candidate nuclear stock tubers, if relevant, and testing to ensure that the candidate nuclear stock plants are free from the specified pests.[3] And if needed, application of virus elimination methods. Microplants shown to be free from the specified pests are designated as nuclear stock.
3. Maintenance of nuclear stock as microplants.
4. Multiplication of nuclear stock in vitro and planting of microplants, under protected conditions to produce plantlets and minitubers (Pre-basic TC class plantlets and seed (mini) tubers).
5. Production of Pre-basic, Basic and Certified category seed tubers.
6. Issue of official certificates (e.g. labels) for Pre-basic, Basic and Certified category seed tubers.

This is mostly covered by UNECE Standard (UNECE, 2021) and the stages are illustrated in Figure 1.

Throughout the whole production, there should be checks to detect and eliminate off-types to maintain the trueness to type for the cultivar.

3.1 | Selection of candidate nuclear stock

New or existing cultivars of potato (*Solanum tuberosum*)[4] are selected as candidate nuclear stock[5] (as tubers or microplants). This starting material should be derived from plants selected visually, for trueness to type for the cultivar and freedom from pests. Starting material may be obtained from certification schemes in other EPPO countries, but material imported from outside the EPPO region should first undergo quarantine testing as described in PM 3/21 *Post entry quarantine for potato* (EPPO, 2019a).

Cultivar identity may be confirmed by 'DNA finger printing' using microsatellite markers or other equivalent method, during the selection of material or during the production of nuclear stocks (Marhadour et al., 2017; Reid et al., 2009, 2011).

3.2 | Production of nuclear stock

The candidate nuclear stock should be kept under confinement in an officially approved micropropagation and insect-proof glasshouse facility, separately from

[2]The NPPO is responsible for regulated pests.

[3]In this standard virus/viroid names refers to the species and are thus italicized.

[4]The scheme may be extended to other tuber-forming species of *Solanum* or their hybrids with *S. tuberosum*.

[5]UNECE use initial stock which is defined as 'initial or nuclear stock refers to the pathogen-tested microplants that form the basis of tissue culture seed potato propagation cycle' (UNECE, 2021).

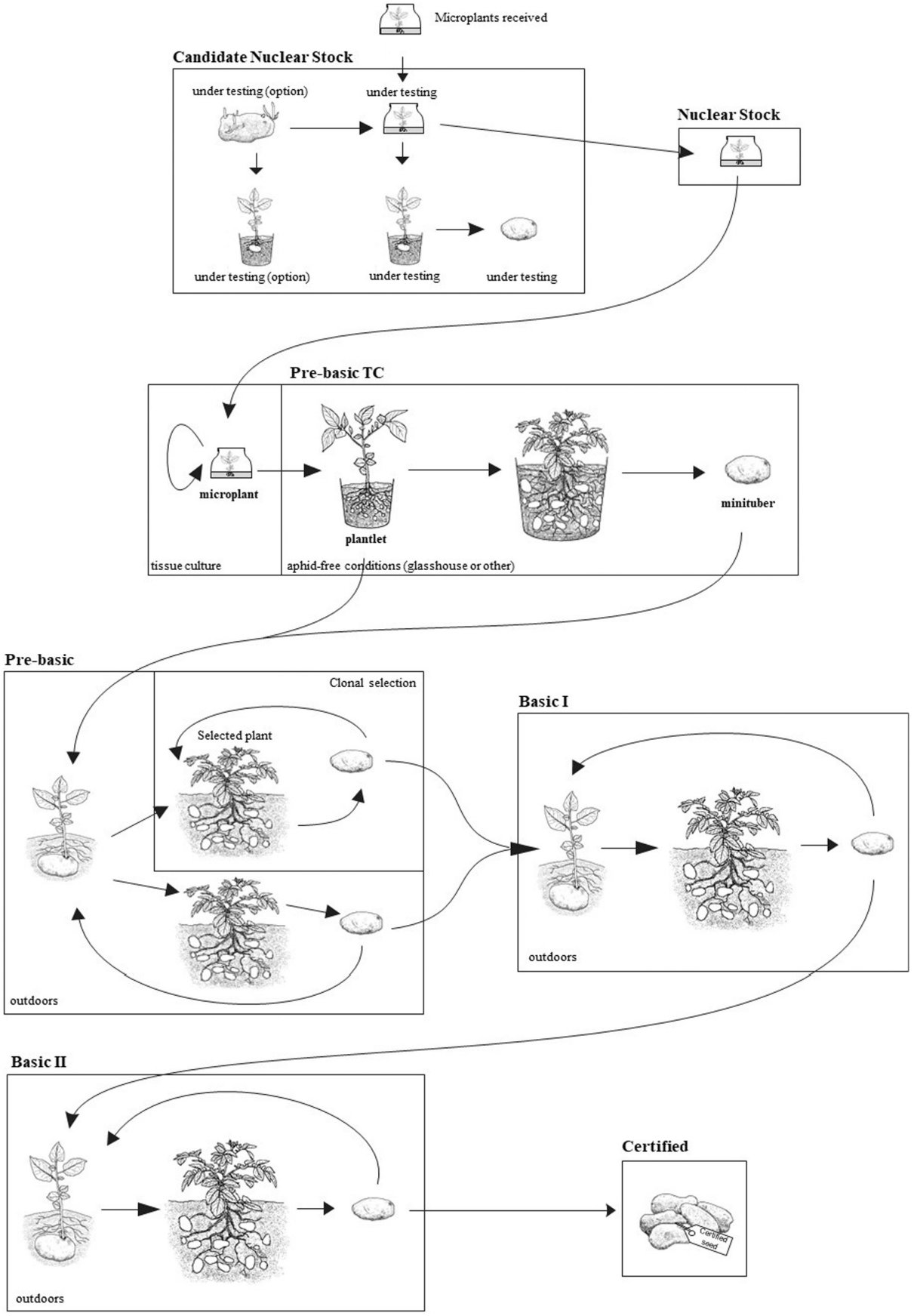

FIGURE 1 Diagram of the stages in the potato certification scheme.

TABLE 1 Tuber-borne potato pests occurring in the EPPO region which are recommended for testing in the scheme.

Pest		Spread in potato fields by
Virus species	Genus	
Potato virus A (PVA)	*Potyvirus*	Aphids
Potato virus M (PVM)	*Carlavirus*	Aphids
Potato virus S (PVS)	*Carlavirus*	Aphids, contact[a]
Potato virus X (PVX)	*Potexvirus*	Contact[a]
Potato virus Y (PVY)	*Potyvirus*	Aphids
Potato leafroll virus (PLRV)	*Polerovirus*	Aphids
Alfalfa mosaic virus (AMV)	*Alfamovirus*	Aphids
Beet ringspot virus (BRSV)	*Nepovirus*	Nematodes (*Longidorus elongatus*)
Cucumber mosaic virus (CMV)	*Cucumovirus*	Aphids
Potato aucuba mosaic virus (PAMV)	*Potexvirus*	Aphids
Potato mop-top virus (PMTV)	*Pomovirus*	Protista (*Spongospora subterranea*)
Potato virus V (PVV)	*Potyvirus*	Aphids
Tobacco mosaic virus (TMV)	*Tobamovirus*	Contact[a]
Tobacco necrosis virus D (TNV-D)	*Betanecrovirus*	Fungi (*Olpidium brassicae* sensu *lato*)
Tobacco rattle virus (TRV)	*Tobravirus*	Nematodes (certain species *Paratrichodorus* and *Trichodorus* spp.)
Tomato black ring virus (TBRV)	*Nepovirus*	Nematodes (e.g. *Longidorus attenuatus*)
Tomato mosaic virus (ToMV)	*Tobravirus*	Contact[a]
Tomato spotted wilt virus (TSWV)	*Orthotospovirus*	Thrips
Viroid species		
Potato spindle tuber viroid (PSTVd)	*Pospiviroid*	Contact[a], aphids (if encapsidated with PLRV)
Bacterial species		
Clavibacter sepedonicus		Contact[a]
Ralstonia solanacearum[b]		Contact[a], water
Dickeya spp.		Contact[a], water
Pectobacterium spp.		Contact[a], water
Phytoplasma species		
'*Candidatus* Phytoplasma solani' (Potato stolbur)		Leafhoppers
Liberibacter species		
Candidatus 'Liberibacter solanacearum'		Psyllids
Nematode species		
Globodera pallida		Soil
G. rostochiensis		Soil

[a]Contact here means contact between plants or with machinery.

[b]*Ralstonia pseudosolanacearum* is not included in the table because it is not present in potato tubers in the EPPO region, but only reported in other hosts (in Germany, in greenhouse, under eradication) and in surface water (in the Netherlands, under surveillance).

the nuclear stock (IPPC, 2019). Any growing medium should be pest free. Material produced by micropropagation should be produced following methods such as those in Appendix 2. A testing programme should be applied to ensure that all material held as individual microplants is free from the pests listed in Table 1, taking into account the risk-based approach for testing nuclear stock described in Appendix 5. Recommended test methods are given in Appendix 3. Depending on the pest, the test may be applied to the candidate nuclear stock plant (leaves or tubers), microplant, or plants and tubers grown from the microplant under confinement in a glasshouse. The testing programme should be designed to minimize the risk of failing to detect a pest because of its uneven distribution or low concentration.

Plants giving negative results in all tests should be transferred to a separate micropropagation facility (see Section 3.3) to become nuclear stock.

Plants giving positive results in any test should be removed immediately, and consideration should be given to retesting other plants in the facility in case of possible cross infection. If no plants of a cultivar are found to be virus free, procedures may be applied to eliminate

infection (Appendix 4). The microplants resulting from virus elimination are considered to be candidate nuclear stock and should be retested for the specified viruses and reassessed for agronomic and cultivar characteristics. It should be noted that if meristem culture is used as the virus elimination procedure, it is also very effective in eliminating fungal and bacterial diseases of potato.

The laboratory handling the candidate potato nuclear stock should not grow other plant material likely to carry potato pests (EPPO, 2019b). However, other plant species may be grown if this is officially permitted, and the pest risks to potato material have been assessed. If identified, the risk should be mitigated by testing this material and by separation in space and time from the candidate potato nuclear stock. Where the laboratory also carries out testing for pests and therefore uses positive controls, measures should be taken to avoid the risk of infecting the candidate potato nuclear stock or plants derived from it. This includes separation of the testing laboratory from areas growing the candidate nuclear stock, separate staff and documented phytosanitary procedures.

3.3 | Maintenance of the nuclear stock

Nuclear stock microplants should be kept in an officially approved micropropagation facility, containing only nuclear stock plants (EPPO, 2019b). Microplants should be maintained in such a way as to avoid the formation of callus tissue or die-back and under conditions to prevent their infestation by pests. The microplants should be stored on a medium without growth hormones. A medium, which restricts plant growth such as Murashige and Skoog (M & S) medium with 3% mannitol is suitable. The microplant cultures may be checked for trueness to type every 2–4 years, for example by growing five or six microplants from each culture in the field and examining the plants visually for trueness to type. Nuclear stock may be maintained indefinitely in tissue culture provided the above requirements are met.

3.4 | Production of seed potatoes (equivalent to EPPO propagation stock)

Seed potatoes should be placed into three categories (Pre-basic, Basic, Certified) and in each category, classes may be defined by the official organization. The different steps of production are subject to official inspections.

3.4.1 | Pre-basic category

In the Pre-basic category two classes may be defined: Pre-basic Tissue Culture (TC) class and Pre-basic seed class (UNECE, 2021).

3.4.1.1 | *Pre-basic TC class (equivalent to EPPO propagation stock I)*

The Pre-basic TC (PBTC) class corresponds to minitubers derived from nuclear stock and meeting the requirements specified in Appendix 5. However, countries may also wish to certify (1) microplants (in vitro) and also (2) plantlets (in vivo) grown from microplants under specified protected conditions. These types of propagation material could be distinguished from PBTC (i.e. minitubers) by specifying them as PBTC microplants and PBTC plantlets. Alternatively these types of propagating material could be distinguished by labelling them as PBTC minitubers, PBTC microplants and PBTC plantlets.

The micropropagation laboratory and minituber production facility, as for nuclear stock, should not grow other plant material likely to carry potato pests, except when this is officially permitted and the pest risks to potato have been assessed and mitigated. Where the laboratory uses positive controls, measures to avoid the risk of infecting microplants or minitubers being produced should be taken (see Section 3.2).

Micropropagation. Laboratories should be officially approved to produce microplants, which may be used for the production of plantlets in vivo for planting, either directly in the field or to produce minitubers. These laboratories should demonstrate a proficiency in aseptic techniques for such production. Laboratories should ensure sterile conditions and maintain a record system that documents the source of the material and the volume of production. PBTC microplants may be produced over an indefinite number of generations and their certification may be based solely on record checks.

Plantlets and minitubers. Plantlets and minitubers should be grown in a suitably designed insect-proof facility isolated from other plant material not derived from tissue culture and from sources of regulated pests (ISPM 33). Additional security can be achieved if the production takes place at a time of year when there is little or no risk of insects entering the facility.

The growing medium should be pest-free. The growing crop should always be kept free from aphids and other pests. The occurrence, development or spread of pests should be prevented by appropriate husbandry practices, and insecticides and fungicides applied as necessary. To avoid infestation by pests the following measures may be used: use of protective clothing; disinfection or change of shoes; use of pest-free soil; use of water free from potato pests (e.g. tap water or rain water). Further measures which may be required are in PM 3/63 (EPPO, 2019c) and ISPM 33 (IPPC, 2019).

Each crop should be officially inspected at least once during the growing season and found to be free from potato pests. The traceability of crops should be recorded so that each PBTC crop is known to be derived from an identified nuclear stock. No further

generations should be permitted for plantlets and for minitubers.

Certification of 'PBTC plantlets and minitubers' will be granted based on records and inspections carried out on the growing crop and additionally on harvested minitubers; and should conform as appropriate to the recommended certification standards in Appendix 5.

Progeny plants grown from PBTC class plantlets and minitubers should not exceed the tolerances for plants not true to type for the cultivar (off-types or incorrect cultivar) and symptoms of virus disease shown in Table A3.

3.4.1.2 | *Pre-basic seed class (equivalent to EPPO propagation stock II)*

Pre-basic seed is seed multiplied in the field directly from PBTC plantlets or minitubers, or Pre-basic seed of prior generations, over a maximum number of field generations set by the official organization.

It is produced under conditions that reduce the risk of virus spread by insects (including the crop being protected with insect proof netting where appropriate, suitably timed insecticide/oil sprays, roguing, early haulm-killing). Precautions should be taken to minimize the spread of mechanically transmitted diseases through hygienic measures and clean equipment. The crop should be planted in soil not known to be infested with *Ditylenchus destructor*, *D. dipsaci*, *Meloidogyne chitwoodi*, *M. fallax* and *Synchytrium endobioticum*. Sampling and testing for these pests will depend on the pest risk and may depend on, for example, previous findings and soil type. The soil should be sampled prior to planting and the samples found free from potato cyst nematodes *Globodera pallida* and *G. rostochiensis* (see point 8 of Appendix 3). The crop/lot should be free from EPPO A2 quarantine pests (see Table A4 of Appendix 5) and pests assessed[6] as fulfilling the minimum criteria for listing as RNQPs, with a zero tolerance ('*Candidatus* Liberibacter solanacearum', *D. destructor* and *Phthorimaea operculella*). Any other national regulations concerning these pests should be respected. General precautions against pests should be maintained. The crop should be inspected during the growing season at times appropriate for the detection of the target pests. The number of inspections will depend on when the crop is exposed to the risk of infection by plant pests, but a minimum of two inspections is recommended.

The traceability of crops should be maintained so that the number of field generations is known (see Section 3.4.4).

The material should conform to the recommended certification standards in Appendix 5. These include virus and off-type tolerances for the progeny produced from the tubers. Compliance with the requirements may be checked, if appropriate, by virus testing of the progeny tubers pre-harvest or post-harvest, usually for common aphid-transmissible viruses. The certifying authority may decide whether there is a need for a pre/post-harvest test according to the risk that the crop has been infected by viruliferous aphids; this depends for example, on factors such as the climate, the cultivar, the proximity to other potato crops, the level of virus infection in the planted seed lot and in the developing crop, the prevalence of aphids early or late in the growing season. Certification will be granted based on records of the inspections/tests performed on the growing crop, of pre/post-harvest tuber tests (if any) and of inspections of harvested tubers intended for marketing.

Progeny plants grown from Pre-basic class seed should not exceed the tolerances for plants not true to type for the cultivar (off-types or incorrect cultivar) and symptoms of virus disease shown in Table A3 of Appendix 5.

Notwithstanding the above, tubers from individually selected plants of the generations of the Pre-basic seed can, under special conditions defined in Appendix 1, be used in place of the initial plantlets or minitubers to start a new cycle of Pre-basic seed. This process has been described as 'clonal selection'.

3.4.2 | Basic seed category (equivalent to EPPO propagation stock III)

Basic seed potatoes are descended directly from Pre-basic category or Basic seed category and are mainly intended for the production of Certified seed potatoes.

After the permitted number of generations of Pre-basic seed class, or if the certification standards for Pre-basic category are not met, the material passes to Basic seed or Certified seed category. The conditions for soil testing, inspection, pre/post-harvest tests and protection against pests during production of Basic category seed are essentially the same as for Pre-basic seed class (see 3.4.1), except that the certification standards are set at a lower level.

The seed may be certified as either Basic I or Basic II according to the minimum requirements given in Appendix 5. However, Basic seed category may be split into more classes, e.g. S, SE, E in the EU, with increasing tolerances up to a maximum tolerance corresponding to the one given for Basic II in Table A3 of Appendix 5, and with a maximum number of field generations for Basic category seed set by the official organization.

Progeny plants grown from Basic category seed should not exceed the tolerances for plants not true to type for the cultivar (off-types or incorrect cultivar) and symptoms of virus disease shown in Table A3 of Appendix 5.

[6]The assessment was performed by EPPO following a 2-year project on RNQPs funded by the European Commission (Picard et al., 2018), or for the preparation of this Standard. Additional pests were assessed as fulfilling the criteria only, for nuclear stock (see Appendix 5).

3.4.3 | Certified seed category (equivalent to EPPO propagation stock IV)

Certified seed potatoes are descended directly from Pre-basic, Basic or Certified seed categories and are intended mainly for the production of potatoes other than seed potatoes. Seed may be classified as either Certified I or Certified II, according to the minimum requirements given in Appendix 5, although countries (e.g. in the EU) may use letters such as A and B to describe these classes.

After the permitted number of generations of Basic seed category, or if the certification standards for propagation of Pre-basic or Basic seed are not met, the material passes to the Certified seed category. The conditions for soil testing, inspection, pre/post-harvest tests and protection against pests during production of Certified seed category are essentially the same as for Basic seed, except that the certification standards are set at a lower level. The number of field generations is also fixed for the Certified seed category.

Progeny plants grown from Certified category seed should not exceed the tolerances for plants not true to type for the cultivar (off-types or incorrect cultivar) and symptoms of virus disease shown in Table A3 of Appendix 5.

3.4.4 | Number of field generations

Each seed category may be certified also according to the number of field generations e.g. FG1, FG2 etc. The maximum number of generations allowed in Pre-basic, Basic and Certified seed categories is decided by the certification authority but, the total for all field generations should not exceed 9.

For all field generations, official checks should be made on cultivar identity and purity and on possible mutations.

3.5 | Administration of the certification scheme

3.5.1 | Monitoring of the certification scheme

An official organization should be responsible for checking the administration and for monitoring of the whole certification scheme. This should include official control of the registered nuclear stock, micropropagation, plantlet and minituber facilities, ensuring that all necessary tests and inspections have been performed during production, and phytosanitary measures have been applied.

The management system, and operating procedures and instructions of the facilities should be documented and be available for auditing by the official organization. Facilities may be accredited to a quality assurance scheme. Official authorisation of a facility to perform diagnostic tests should follow PM 7/130 *Guidelines on the authorization of laboratories to perform diagnostic activities for regulated pests* or may be met by facility accreditation to ISO 17025 for the required tests (see PM 7/98 *Specific requirements for laboratories preparing for accreditation for a plant pest diagnostic activity*).

3.5.2 | Control on the use and status of certified material

Throughout the certification scheme, the origin of each plant should be traceable so that any problems of health or trueness to-type may be traced by the official organization.

Plants certified under the scheme should carry an official certificate (e.g. a label) indicating the official organization in charge of certification, and where appropriate, the plant producer (or producer number), the crop number, the category, the class, the cultivar, the size and weight, the date of container closing and the number of field generations.

ACKNOWLEDGEMENTS

This Standard was originally prepared by the Panel on Certification for Seed Potatoes and first approved in 1999. A first revision was prepared in 2022 by Mr C. Jeffries (SASA, GB) and discussed in an expert working group composed of Ms H. Esan Günaçti (BCRI, TR), Mr C. Jeffries, Ms H. Kortemaa (Finish Food Authority), Mr N. Ponserre (SEMAE, FR) and Mr A. Toussaint (NAK, NL). The draft revised Standard was reviewed by the Panel on Phytosanitary Measures for Potato.

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How to cite this article: EPPO (2023) PM 4/28(2) Certification scheme for seed potatoes. *EPPO Bulletin*, 53, 460–475. Available from: https://doi.org/10.1111/epp.12950

APPENDIX 1 - DETAILS ON THE SELECTION OF MOTHER PLANTS IN CLONAL SELECTION

Clonal selection is a system of seed potato propagation that starts by selecting, healthy looking, high yielding and true to type mother plants from the first three field generations of Pre-basic seed class. These mother plants are harvested separately and propagated as separate clones to produce Pre-basic seed for a maximum of 4 field generations (PB1—PB4).

Selected mother plants should be free from pests as listed in Appendix 5 for nuclear stock, based on inspection or testing.

The first generation of new clonal stock (progeny of selected mother plants) should be inspected to check compliance with the requirements for PB 1 seed potato category as provided in Tables A3 and A4 in Appendix 5.

After the permitted number of field generations for Pre-basic seed class, or earlier if it fails to meet the specified tolerances for this class, Pre-basic seed is downgraded to Basic or Certified category seed.

APPENDIX 2 - EXAMPLE OF A MICROPROPAG ATION METHOD FOR POTATO

Material submitted as tubers should be washed, surface-sterilized in 0.3% available chlorine for 15 min and incubated in the dark to produce sprouts which are 3–7 cm long. Sprouts should also be surface-sterilized and rinsed in sterile water before excising the axillary buds. These are placed on a growing medium, such as M & S medium with 30 g L^{-1} sucrose and 8 g L^{-1} Oxoid no. 3 agar. The subsequent microplants should be subdivided into nodal segments every 4–6 weeks and transferred to fresh medium. All in vitro work should be done using aseptic techniques in sterile air cabinets. Cultures should be incubated at 18–20°C under cool-white-fluorescent tubes at a 16 h day-length. Microplants should be inspected regularly for signs of bacterial or fungal infection, particularly in the first 5–10 days after subculture. Infection by endogenous bacteria may be seen as slight milky streaming at the base of the explant or from the roots. These bacteria maybe eliminated using procedures described in Appendix 4.

APPENDIX 3 - GUIDELINES ON TESTING PROCEDURES

The tests presented in this Standard may be adjusted to suit individual laboratories and to reflect developments in methods, if they are adequately validated or verified, and the uncertainty of detection is not increased.

1. Testing for viruses in candidate material

Tests are performed on candidate material in confinement. Matrices to be tested may be leaves of a candidate nuclear stock plant grown from a tuber, microplants or leaves of potato plants grown from candidate microplants. Testing the candidate material several times e.g. microplants and plants grown from microplants, may reduce the risk of failing to detect a virus. For microplants, tests should be done on microplant subcultures 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, *Potato mop-top virus* (Nisbet et al., 2004). Examples of the testing programmes for candidate material received as tubers or microplants for post-entry quarantine are given in PM 3/21 (EPPO, 2019a).

ELISA

ELISA is commercially available for many of the viruses that affect potato, particularly for PLRV, PVA, PVM, PVS, PVX and PVY. Each plant of the candidate material should be tested separately. Although antibodies should be used according to the manufacturers' instructions, these may be modified if the modifications are verified or validated. General ELISA instructions are described in PM 7/125 *Diagnostic protocol for ELISA tests for viruses* (EPPO, 2015).

Use of indicator plants

The potato viruses covered by the scheme (except PLRV) can be detected by sap inoculation to a range of suitable indicator plants (see Standard PM 7/153 Mechanical inoculation of test plants; EPPO, 2022e). Table A1 gives an example of the use of the following six indicator plants, *Chenopodium quinoa*, *Nicotiana benthamiana*, *N. glutinosa*, *N. occidentalis* P-1, *N. tabacum* cv. White Burley and *Phaseolus vulgaris* cv. The Prince. Other combinations that allow the detection of all the viruses may also be used (EPPO, 2019a). Screening with these indicator plants will detect potato viruses without necessarily identifying them, including PVA, PVM, PVS, PVX and PVY, which are tested for by ELISA in any case. It should be noted that some isolates of PVS can be detected by *Chenopodium quinoa*, but that *C. murale* can detect more isolates. The reliability of bioassays may be affected by several factors and some viruses may require special inoculation buffers.

Molecular tests

Instead of using ELISA and indicator plants for virus detection, molecular tests described in PM 3/21 (EPPO, 2019a) may be used to ensure detection off all the viruses listed in Table A1 apart from *Cucumber mosaic virus*.[7] High-throughput sequencing (also known

[7]Test for this virus is described in Kimaru et al. (2020).

TABLE A1 Indicator plants for potato viruses.

Virus species	Indicator plants					
	Chenopodium quinoa	*Nicotiana benthamiana*	*N. glutinosa*	*Nicotiana occidentalis* cv. – P1	*N. tabacum* cv. White burley	*Phaseolus vulgaris* cv. The prince
Potato virus A (PVA)				X	X	
Potato virus M (PVM)				X		X
Potato virus S (PVS)	X[a]			X		
Potato virus X (PVX)		X	X	X	X	
Potato virus Y (PVY)		X		X	X	
Potato leafroll virus (PLRV)						
Alfalfa mosaic virus (AMV)	X			X		X
Beet ringspot virus (BRSV)			X			
Cucumber mosaic virus (CMV)	X					
Potato aucuba mosaic virus (PAMV)			X	X		
Potato mop-top virus (PMTV)	X	X				
Potato virus V (PVV)		X		X		
Tobacco mosaic virus (TMV)			X	X		
Tobacco necrosis virus D (TNV-D)	X			X	X	
Tobacco rattle virus (TRV)	X					X
Tomato black ring virus (TBRV)	X			X	X	
Tomato mosaic virus (ToMV)			X	X	X	
Tomato spotted wilt virus (TSWV)		X	X	X	X	

Note: The range of indicator plants given here is an example of the use of a limited number of species to detect all potato viruses (apart from PLRV); other suitable combinations may also be used.

[a]*Chenopodium quinoa* seems to vary in its susceptibility to PVS isolates and does not detect some. Whereas *C. murale* will detect all isolates. ELISA should allow the detection of all isolates.

as next-generation sequencing) may also be considered as an equivalent test (EPPO, 2022d; Olmos et al., 2018).

2. Pre-/post-harvest tuber tests of the direct progeny

There are two main post-harvest tuber tests: growing-on of tuber eyeplugs after breaking of tuber dormancy followed by ELISA on leaves of progeny plants; and direct real-time RT-PCR testing of tubers without necessarily breaking dormancy. Such tests are usually done for viruses which are commonly known to be present such as PLRV, PVA, PVX and PVY. However, testing for other viruses may be performed depending on the pest risk obtained for example from monitoring and survey results. Pre-/post-harvest examination of growing plants may also be done visually in the fields or greenhouses, with confirmation by laboratory testing as required.

Growing-on/ELISA is relatively simple to perform, low cost and robust. However, it does require glasshouse space and it has a relatively long testing time, mainly for breaking tuber dormancy and then growth of the eyeplugs (3–4 weeks). On the other hand, although real-time RT-PCR reagents are more costly than ELISA, the method can be applied directly to dormant tuber material on larger tuber bulks reducing costs and shortening the testing time to a few days.

Testing is done on 100–400 tubers randomly selected from each crop. The number of tubers in a sample depends on the tolerance required, the confidence level used and the heterogeneity of the lot. Confidence limits for testing of individual tubers and bulked samples to determine whether the virus incidence of seed potato stocks falls within tolerance levels, are described by the UNECE (2021). Seedcalc8″ software or equivalent from the International Seed Testing Association (ISTA) (https://www.seedtest.org/upload/cms/user/Seedcalc8.ziph) can also be used.

Growing-on test

Lacomme et al. (2015) describes a procedure which could be used for a growing-on test. From each tuber remove a rose-end eyeplug using, for example, a melon scoop. Soak eyeplugs in 1 µM gibberellic acid for 10 min to encourage sprout growth, drain and store eyeplugs overnight at room temperature to dry. Plant in sterilized compost or a soil-less medium and incubate at 20–25°C, 16 h photoperiod. After 3–4 weeks plants should be about 20–30 cm tall and ready for testing. Testing may be carried out on

bulked samples of four eye plugs. Harvest one leaf (one compound leaf near the apex) per plant and conduct ELISA according to instructions from the manufacturer of the antibodies/ELISA kit. Store the tubers and keep all the plants until testing is complete.

Direct tuber test

Lacomme et al. (2015) provides a procedure which could be used. Remove excess soil (by brushing off or briefly washing the tubers). Peel the skin (~2–3 mm thick) from the rose and heel end (stolon end) of each of 10 tubers. Place the peelings in a homogenization bag. Store at −20°C until homogenization and RNA extraction. Extract and test for the viruses required. Tissue from more than 10 tubers may be bulked if validated.

Extraction methods and real-time RT-PCR testing details for PLRV, PMTV, PVA, PVX, PVY and TRV are provided in Lacomme et al. (2015). References for primer and probe details for these and other viruses and internal controls are provided in Table A2.

3. Testing for Potato spindle tuber viroid (PSTVd)

The test for PSTVd (genus *Pospiviroid*) is applied to leaves of candidate nuclear stock plants grown from tubers or microplants under confinement in a glasshouse. The test can be done by hybridization with a digoxigenin-labelled cRNA probe, conventional RT-PCR or real time RT-PCR (EPPO, 2004, 2021; IPPC, 2015). Tests may be Pospiviroid specific or of higher specificity to detect PSTVd and a reduced number of pospiviroids. Since none of the tests are specific for PSTVd, sequencing of the PCR product is required for confirmation and identification.

4. Testing for *Clavibacter sepedonicus*

The test is applied to a potato tuber (core extracted from the stolon end), either the candidate tuber itself or a daughter tuber of a plant grown from a microplant of candidate material under confinement in a glasshouse. Microplants may also be tested. Tests include immunofluorescence, PCR (conventional and real time) and fluorescence in situ hybridization (FISH). These are described in PM 7/59 *Diagnostic protocol for Clavibacter sepedonicus* (EPPO, 2022b). When two tests (based on different biological principles e.g. molecular/serological or two molecular tests targeting different parts of the genome) are positive *C. sepedonicus* is considered to be detected in the sample. For critical cases, isolation should be performed followed by identification tests. Details on the testing procedure are given in PM 7/59 (EPPO, 2022b).

5. Testing for *Dickeya* spp. and *Pectobacterium* spp.

In Europe pectolytic bacteria of the *Dickeya* and *Pectobacterium* genera have been identified as causing blackleg and slow wilt of potato (van der Wolf et al., 2017). The emergence of numerous distinct new and more aggressive *Dickeya* and *Pectobacterium* species since 2010 (Czajkowski et al., 2015; Humphris et al., 2015; van der Wolf et al., 2017) has forced seed potato growing countries to reduce the tolerance for these pests in their certification schemes, including tests of the nuclear stock. Several diagnostic tests are available for the detection and identification of *Dickeya* and *Pectobacterium* on different plant and environmental matrices, e.g. molecular tests, proteomic analysis, fatty-acid analysis, isolation and pathogenicity tests. An EPPO protocol for the diagnosis of *Dickeya* and *Pectobacterium* spp. mainly focusing on potato has just been published (EPPO, 2023).

6. Testing for *Ralstonia solanacearum*

The test is applied to a potato tuber (core extracted from the stolon end), either the candidate tuber itself or a daughter tuber of a plant grown from a microplant of candidate material, under confinement in a glasshouse. Microplants may also be tested. Tests include selective plating on SMSA medium, immunofluorescence, and PCR (conventional and real time). These are described in PM 7/21 *Diagnostic protocol for* Ralstonia solanacearum, R. pseudosolanacearum and R. syzygii *(*Ralstonia solanacearum *species complex)* (EPPO, 2022a). When two tests (based on different biological principles e.g. molecular/serological, or two molecular tests targeting different parts of the genome) are positive *R. solanacearum* is considered detected in the sample. For critical cases, isolation should be performed followed by identification tests. When isolation is performed and colonies with typical morphology are detected, two identification tests should be performed to confirm the identification. Details on the testing procedure are given in PM 7/21 (EPPO, 2022a).

7. Testing for '*Candidatus*' spp.: '*Ca*. Liberibacter solanacearum' and '*Ca*. Phytoplasma solani'

For '*Ca*. Phytoplasma solani' (Potato stolbur) 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 candidate nuclear stock tubers (heel-ends) or microplants should be tested 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 (EPPO, 2019a). Samples may be tested for phytoplasmas by using universal phytoplasmas primers. ISPM 27 DP12: *Phytoplasmas* and EPPO Standard PM 7/133 *Diagnostic protocol for the Generic detection of phytoplasmas* provide details of nucleic acid extraction and universal conventional nested-PCR and real-time PCR tests (EPPO, 2019a).

TABLE A2 Examples of real-time RT-PCR primers and probes used by various laboratories for the post-harvest detection of potato viruses.

Virus species	Forward primer 5′–3′	Reverse primer 5′–3′	Probe	Reference	Name of laboratory
PLRV	PLRV-F	PLRV-R	PLRV-Probe	Boonham et al. (2009)	SASA[a] NAK
PMTV	PMTV-1948F	PMTV-2017R	PMTV1970-Probe	Mumford et al. (2000)	SASA[a] NAK
PVA	PVA-FWD	PVA-REV	PVA-Probe	Lacomme et al. (2015)	SASA[a]
PVA	PVA 102-2 FP	PVA 102-2 RP	PVA 102-2 probe	Agindotan et al. (2007)	NAK
PVS	PVS-1 for	PVS-1 rev	PVS-1 probe	Mortimer-Jones et al. (2009)	NAK
PVX	PVX 101-2 FP	PVX 101-2 RP	PVX 101-2 probe	Agindotan et al. (2007) Same primers and probe sequences also used by Mortimer-Jones et al. (2009)	SASA[a]
PVX	PVX 1F PVX 2F	PVX 1R PVX 2R	PVX Probe	Boonham et al. (2009)	NAK
PVY	PVY 411-F	PVY 477-R	PVY-Probe	Boonham et al. (2009)	SASA[a] NAK
TRV	TRV-1466F	TRV-1553R	TRV-1489-Probe	Mumford et al. (2000)	SASA[a] NAK
COX Internal control	COX-F	COX-RW	COXSOL1511T-Probe	Boonham et al. (2009)	SASA[a]
Chloroplast ATP synthase beta-subunit	MultiPot-F	MultiPot-R	MultiPot-P	Massart et al. (2014)	NAK

[a]ISO 17025 accredited test in this laboratory.

'*Candidatus* Liberibacter solanacearum'

'*Ca*. Liberibacter solanacearum' can be detected using specific methods, such as real-time PCR. Tests are provided in ISPM 27 DP 21: 'Candidatus *Liberibacter solanacearum*' and PM7/143 *Diagnostic protocol for* 'Candidatus *Phytoplasma solanacearum*' (EPPO, 2020a).

8. Testing soil for potato cyst nematodes *Globodera pallida* and *Globodera rostochiensis*

Sampling details are provided in Appendix 3.1 of PM 9/26 *National regulatory control system for* Globodera pallida *and* G. rostochiensis (EPPO, 2000).

The soil samples are then processed in the laboratory. Extraction details are provided in PM 7/119 *Diagnostic protocol for Nematode extraction* (EPPO, 2013a).

Identification of *G. pallida* and *G. rostochiensis* may be done by morphological and/or molecular methods, as described in PM 7/40 *Diagnostic protocol for* Globodera rostochiensis *and* Globodera pallida (EPPO, 2022c).

9. Testing soil, roots and tubers for *Meloidogyne chitwoodi* and *Meloidogyne fallax*

Sampling details for soil and potatoes are given respectively in PM 9/17 *National regulatory control system for* Meloidogyne chitwoodi *and* Meloidogyne fallax (EPPO, 2013b) and in PM 3/69 *Phytosanitary procedure for* Meloidogyne chitwoodi *and* M. fallax: *sampling potato tubers for detection* (2019d).

Guidance on extraction is given in PM 7/119 *Diagnostic protocol for nematode extraction* (EPPO, 2013a).

Identification of *M. chitwoodi* and *M. fallax* may be done by morphological and/or molecular methods, as described in PM 7/41 *Diagnostic protocol for* Meloidogyne chitwoodi *and* Meloidogyne fallax (EPPO, 2016).

APPENDIX 4 - EXAMPLES OF PROCEDURES FOR ELIMINATING VIRUSES

Virus-infected material submitted as tubers should be washed and surface-sterilized as described in Appendix 2 before use of one of two possible methods to eliminate the virus.

In the first method (MacDonald, 1973), the tuber is incubated in a humid chamber at 32–35°C until sprouts are produced. Sprouts are then removed, surface-sterilized in 0.3% available chlorine for 15 min and washed in sterile water. The apical meristem (150–200°µm) is excised, placed on M & S medium and incubated at 18–20°C under cool-white, fluorescent tubes at a 16 h day-length. The meristems are subcultured onto new M & S medium at intervals of 4–6 weeks until a plantlet is produced.

In the second method (Jeffries, 1998), microplants are produced as described in Appendix 2. Nodal sections from the microplants are transferred to M & S medium containing 20 mg L^{-1} ribavarin. These nodes are incubated for 10 days at 18°C under lights at a 16-h day length to allow the microplants to grow and root. The cultures are then subjected to an alternating temperature regime

of 40°C for 4h followed by 25°C for 4h. After 4weeks, each microplant is subdivided into nodes. The first node beneath the tip is grown on for virus testing, and the second node is transferred to fresh ribavarin-amended M & S medium and the treatment repeated as described above until plantlets are found to be free from viruses.

APPENDIX 5 - RECOMMENDED CERTIFICATION STANDARDS FOR SEED POTATOES

Certification will be granted based on records of the tests/inspections performed on the growing crop, of pre/post-harvest tuber tests (if applicable) and of inspections

TABLE A3 Suggested minimum tolerances recommended for the seed potato categories, Pre-basic, Basic seed and Certified seed.

	Seed potato categories					
	Pre-basic		Basic seed		Certified seed	
	Pre-basic TC (plantlets/ minitubers)	Pre-basic seed	I	II	I	II
Growing crop inspection	As a % of plants					
Symptoms of virus infection	0	0.1	0.2	0.8	2	6
Blackleg (*Dickeya* spp., *Pectobacterium* spp.)	0	0	0.5	1	1.5	2
'*Candidatus* Liberibacter solanacearum' non-solanaceous/ European haplotypes[a]	0	0	0		0	
Off-types[b] and different cultivar	0	0.01	0.25		0.5	
Direct progeny[c]	As a % of plants					
Symptoms of virus infection	0	0.5	1	4	8	10[d]
Off-types[b]	0	0.01	0.25		0.5	
Lot tolerances: tuber diseases and defects[e]	As a % of total weight					
*Dry and wet rot[f]	0	0.2	1 (0.5 wet rot)	1 (0.5 wet rot)		
*Black scurf (*Thanatephorus cucumeris*=*Rhizoctonia solani*)	0	1 (sa[g]>10%)	5 (sa>10%)		5 (sa>10%)	
*Common scab (*Streptomyces* spp.)	0	5 (sa>33%)	5 (sa>33%)		5 (sa>33%)	
*Powdery scab (*Spongospora subterranea*)	0	1 (sa>10%)	3 (sa>10%)		3 (sa>10%)	
'*Candidatus* Liberibacter solanacearum' non-solanaceous/ European haplotypes[a]	0	0	0		0	
Ditylenchus destructor	0	0	0		0	
Phthorimaea operculella	0	0	0		0	
*External defects e.g. misshapen, cracks	3	3	3		3	
Earth and extraneous matter	1	1	2		2	
Visual necrosis[h]	0	1[i]	Not specified		Not specified	
*Shrivelled tubers, including dehydration caused by silver scurf (*Helminthosporium solani*)	0	0.5	1		1	
Chilling injury	0	2	2		2	
Pest damage (e.g. slugs, wireworms, tuber moth, flea beetles): Tubers with more than 10 holes or with more than 3 holes of 5mm or more in depth are countable	0	4	4		4	
Total tolerance (As a % of total weight, for items marked with a '*')	3	5	6		6	

Note: Pests locally present in the region and recommended for regulation by EPPO as quarantine pests (EPPO A2 pests) have a zero tolerance. For these pests see Table A4.

[a]See Appendix IV of Standard PM 9/25 Control system against '*Candidatus* Liberibacter solanacearum' haplotypes present in the EPPO region (EPPO, 2020b).

[b]Including malformation caused by plant protection products.

[c]The direct progeny tolerances also apply to pre/post-harvest tuber tests.

[d]Severe virus symptoms only.

[e]Only applies when tubers are to be marketed.

[f]Includes *Alternaria* spp., *Athelia rolfsii*, *Boeremia* spp., *Fusarium* spp., *Geotrichum candidum*, *Helicobasidium brebissonii*, *Pectobacterium* spp., *Phytophthora erythroseptica*, *P. infestans*, *Pythium* spp., *Sclerotinia minor* and *S. sclerotiorum*.

[g]sa, surface area affected.

[h]"Visual necrosis" means necrotic spots, arcs or rings within or on the surface of the tuber. These symptoms are often (but not exclusively) caused by virus infection.

[i]Provided not more than 0.5% surface necrosis.

of harvested tubers. The inspector will verify that the requirements described below are fulfilled.

Nuclear stock

The micropropagation facilities should satisfy official requirements. Records should show that for all nuclear stock microplants the following pests have not been detected by official testing or are derived from tested materials

- For EPPO quarantine pests: PSTVd, Clavibacter sepedonicus and Ralstonia solanacearum[8]
- For pests considered as fulfilling the criteria for listing as regulated non-quarantine pests (RNQPs) for potato nuclear stock by EPPO following a 2-year project on RNQPs funded by the European Commission (Picard et al., 2018) and included in the UNECE Standard S-1: PLRV, PVA, PVM, PVS, PVX, PVY, *Dickeya* spp. and *Pectobacterium* spp., '*Candidatus* Liberibacter solanacearum' and '*Ca*. Phytoplasma solani'.

If the official organization has determined that the viruses AMV, BRSV, CMV, PAMV, PMTV, PVV, TBRV, TMV, TNV-D, TRV, ToMV and TSWV,[9] and other pests which may infect potato in the EPPO region (see Jeffries & Lacomme, 2018 for virus pest list), may pose a risk to the production of 'pest-free' nuclear stock, then nuclear stock, or material from which it derives, should also be tested for these viruses. The official organization should monitor publications for reports of new pests infecting potato and revise their testing of nuclear stocks appropriately.

No microplants should show any symptom of fungal, bacterial or viral diseases. If this condition is not met, the material should not be accepted as nuclear stock.

Pre-basic TC class (tissue culture)

In the case of micropropagation, the micropropagation facilities should satisfy official requirements. In the case of plantlet and minituber production, all plants and tubers should be free from pests and from any symptoms of attack by pests. The percentage of minitubers showing physical defects and soil and extraneous matter should not exceed 3% and 1% respectively (see Table A3). If these conditions are not met at the time of the growing season inspection and tuber inspection, certification at this class will be refused.

Pre-basic seed class, Basic and Certified seed categories

Records should show that the plot in which the material is planted is free from *Globodera pallida* and *G. rostochiensis* and is not known to be infected with *Ditylenchus destructor*, *D. dipsaci*, *Meloidogyne chitwoodi*, *M. fallax* and *Synchytrium endobioticum*. The crop/lot should be free from EPPO A2 quarantine pests (see Table A4) and pests assessed as fulfilling the minimum criteria for listing as RNQPs, with a zero tolerance ('*Candidatus* Liberibacter solanacearum', *D. destructor* and *Phthorimaea operculella*).

TABLE A4 Pests subject to a 0% tolerance for all seed potato categories for crop/lot inspection since these are recommended for regulation as quarantine pests and locally present in the EPPO region (EPPO A2 pests).

Bacteria
'Candidatus Phytoplasma solani' (stolbur)
Clavibacter sepedonicus
Dickeya dianthicola
Ralstonia solanacearum
Nematodes
Ditylenchus dipsaci
Globodera pallida
Globodera rostochiensis
Meloidogyne chitwoodi
Meloidogyne enterolobii
Meloidogyne fallax
Insects
Epitrix cucumeris
Epitrix papa
Leptinotarsa decemlineata
Tecia solanivora
Viruses
Pepino mosaic virus
Potato spindle tuber viroid
Tomato chlorosis virus
Tomato ringspot virus
Tomato spotted wilt virus
Tomato yellow leaf curl virus
Fungi
Synchytrium endobioticum

Note: For EPPO A1 potato pests see EPPO Standard PM 8/1(2) *Potato. Commodity-specific phytosanitary measures* (EPPO, 2017) or https://www.eppo.int/ACTIVITIES/plant_quarantine/A1_list for an up to date list.

Any other national regulations concerning the pests mentioned here should have been respected, as well as any phytosanitary requirements of other countries to which material from the certification scheme may be exported. The incidence of other pests and disorders in the growing crop and on inspection of tubers intended for marketing should not exceed the tolerances given in Table A3.

[8]Note that, for more practicality, '*Candidatus* Liberibacter solanacearum' (Solanaceae haplotypes, i.e. non-European haplotypes, such as haplotypes A and B) are not included in this list, but included with other haplotypes in the second bullet point. Testing for the presence of the different haplotypes (European or non-European) may be performed simultaneously.

[9]All these viruses, except BRSV, had also been considered as fulfilling the criteria for listing as RNQPs for potato nuclear stock by EPPO following the RNQP Project (Picard et al., 2018). BRSV was not discussed during the project since it had been described as a strain of TBRV. However, it is now characterized as a distinct virus species.

The results of pre/post-harvest tuber tests, if applicable, should not exceed the direct progeny tolerances given in Table A3. If these conditions are not met, certification at this grade will be refused.

APPENDIX 6 - EXAMPLES OF PROCEDURES FOR ELIMINATING ENDOGENOUS BACTERIA

Method A

For *Paenibacillus* spp. subculturing a very small nodal segment and repeating this subculturing a number of times may successfully eliminate *Paenibacillus* spp. However, it can be very time consuming sometimes taking 4 or more subcultures at 4-week intervals to achieve elimination and the method B below may be more appropriate.

Method B

Method A for *Paenibacillus* spp. may not be successful for all bacteria e.g. *Curtobacterium* spp. and therefore the following protocol using an antibiotic/antimycotic supplemented medium may be used:

1. Prepare antibiotic/antimycotic supplemented M & S medium as follows. Add sucrose 30 g, Technical agar No 3 8 g, and M & S medium without growth regulators 1 packet to distilled /deionized water 1000 mL and autoclave. After cooling to about 50–55°C add antibody/antimycotic preparation AAM (Sigma Aldrich A5955).
2. Subculture 5–10 nodal segments onto AAM (1 node per Universal tube) and incubate for 2 months at about 18–20°C 16 h light until there has been about 5–10 mm or more extension growth from the node. Growth is severely inhibited, and no roots may be formed, but this may be cultivar dependant.
3. Closely observe the stem base for bacterial streaming or cloudiness during this period. Discard affected cultures.
4. For the remaining plants subculture an apical nodal segment from each of at least 3 microplants onto M & S medium and incubate at 18–20°C 16 h light.
5. Closely observe the stem base for bacterial streaming or cloudiness particularly within the first 10 days. Discard affected cultures (or if there are no unaffected cultures repeat culturing on AAM).
6. Once plants have reached a sufficient size (5–10 cm) subculture.
7. Test the stem base for bacterial infection by crushing the stems and roots and streaking onto nutrient medium.
8. Discard all subcultures from stem bases showing infection after plating onto nutrient medium.
9. Continue subculturing non-affected cultures closely observing the stem base for bacterial streaming or cloudiness particularly within the first 10 days. No contamination should be present at this stage.
10. As appropriate continue to test stem bases of subcultures by crushing the stems and roots and streaking onto nutrient medium.