



NAPPO Regional Standard for Phytosanitary Measures (RSPM)

RSPM No. 3 Requirements for Importation of Potatoes into a NAPPO Member Country

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Review

NAPPO Standards for Phytosanitary Measures are subject to periodic review and amendment. This standard was last reviewed in October 2003. The next review date for this standard is October 2008. A review of any NAPPO Standard may be initiated at any time upon the request of a NAPPO member country.

Endorsement

This Standard was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on October 22, 2003.

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Implementation

See the attached Implementation Plan.

Amendment Record

Amendments to this Standard will be dated and filed with the NAPPO Secretariat. The most recent version will be posted on the NAPPO website at: www.nappo.org/stds_e.htm

Distribution

This standard is distributed by the Secretariat of the NAPPO within NAPPO, including Sustaining Associate Members and Industry Advisory Groups, to the FAO IPPC Secretariat, and to the Administrative Heads of the Regional Plant Protection Organizations (RPPOs).

Introduction

Scope

This standard applies to seed and fresh market potatoes (tablestock and potatoes for processing) as a pathway for the introduction of regulated pests. Other pathways for the same pests might exist, but they are outside the scope of this standard. Quality characteristics and genetically modified potato cultivars also are outside the scope of this standard. This standard establishes potato trade requirements 1) among the NAPPO member countries and 2) between the individual NAPPO member countries and trading partners external to the NAPPO region. The goal is to reduce the probability of introduction of regulated pests to an acceptable level, while facilitating trade. The objectives of this standard are to:

- Prevent the introduction of quarantine pests into the NAPPO region;
- Prevent spread of quarantine pests already established within the NAPPO region;
- Prevent economically unacceptable impacts associated with regulated non-quarantine pests and
- Facilitate equitable and orderly trade.

References

The United Nations Food and Agricultural Organization's (FAO) International Standards for Phytosanitary Measures (ISPM) numbers 1-17 apply in whole or part to the application of this standard. As relevant, future ISPMs also may apply.

Definitions, Abbreviations and Acronyms

The meaning of all relevant definitions, abbreviations, and acronyms contained in ISPM No. 5, "Glossary of phytosanitary terms 2002" apply to this standard. In addition, the following terms apply:

ELISA	Enzyme Linked Immuno-Sorbent Assay. (NAPPO, 2002)
germplasm	Plants intended for use in breeding or conservation programs. (NAPPO, 2002)
microplantlet	Plantlet produced <i>in vitro</i> on a defined medium. (NAPPO, 2002)
microtuber	A tuber produced <i>in vitro</i> . (NAPPO, 2002)
minituber	A tuber produced in a protected environment from pathogen-free parent material. (NAPPO, 2002)
parent material	<i>In vitro</i> , pathogen-free propagules used to increase a clone of potatoes for production of commercial quantities of microplantlets, microtubers, or minitubers. (NAPPO, 2002)
PCR	Polymerase Chain Reaction. (NAPPO, 2002)
pest risk	That level and probability of harm a plant pest could cause in an endangered area, which a national plant protection organization identifies by the performance of a pest risk analysis or other internationally acceptable plant risk assessment process. (NAPPO, 2002)
potatoes	Any wild or commercially produced plant, tuber, or other part of <i>Solanum tuberosum</i> , including all closely related species and cultivars. (NAPPO, 2002)
propagule	Any plant part used for asexual propagation. (NAPPO, 2002)

propagative plant material	Plants or plant parts for planting or multiplication. (NAPPO, 2003)
quality assurance system	A planned and regularly monitored framework of controls, based on documented procedures, which is applied to critical operational activities in the production of potatoes or the performance of a service (e.g. diagnostics) or activity (e.g. the conduct of a test) to ensure operational efficiency and reliable compliance with specified quality standards. (NAPPO, 2002)
RFLP	Restriction Fragment Length Polymorphism. (NAPPO, 2002)
RT-PCR	Reverse Transcription-Polymerase Chain Reaction. (NAPPO, 2002)
risk management options	Risk reduction actions that may be selected, alone or in combination, to reduce identified pest risk to an acceptable level. (NAPPO, 2002)
seed potato certification	An officially adopted scheme for the production of potato propagative materials that meet prescribed requirements for pest freedom and varietal purity. (NAPPO, 1995). (NAPPO, 2002)
soil	The loose surface material of the earth in which plants grow, in most cases consisting of disintegrated rock with an admixture of organic material. (Soil). (NAPPO, 2002)

Outline of Requirements

This standard identifies the regulated pests of potato based on NAPPO member country quarantine, official seed potato certification program and other regulatory requirements in effect at the time of this revision.

Describes pest risk management options (Section 3) and displays these measures by commodity and by pest (Tables I and II). Pre-shipment testing requirements for PVY^N are specified in Appendix 5.

Describes risk management methods (Sections 4 and 5). Establishes a documentation standard (Section 5).

General Requirements

1. Pests Regulated by NAPPO Member Countries

The pests listed in Appendix 1 are regulated pests for the NAPPO region. The list was compiled from the potato quarantine pest lists and the lists of pests regulated under the terms of seed potato certification programs in the NAPPO member countries.

2. Pest Risk Analysis Standard

The imposition of phytosanitary measures should be based on the results of pest risk analyses performed in accordance with ISPM 11, “Pest Risk Analysis for Quarantine Pests”. Risk will vary among the kinds of potato products including seed potatoes, tablestock, and potatoes for processing.

3. Determination of Appropriate Risk Management and Phytosanitary Measures

Subsection 3.3 describes specific pest risk management methods. Phytosanitary officials may

select available risk management measures to develop a program for reducing the identified risk of introducing quarantine pests to an acceptable level. Depending on the risk associated with a particular commodity and the degree of protection provided by the risk management measures, one or more of the measures may be selected and combined to create a “Systems Approach” for managing the risk as briefly described in subsection 3.2 and in detail in ISPM 14, “The Use of Integrated Measures in a Systems Approach for Pest Risk Management”.

The "managed risk" principle is to be applied in the administration of this standard. ISPM 1, “Principles of Plant Quarantine as Related to International Trade” explains that, "Because some risk of introduction of a quarantine pest always exists, countries shall agree to a policy of risk management when formulating phytosanitary measures." ISPM 1 adds, “countries should decide what level of risk is acceptable to them”. The acceptable level of risk may be expressed in a number of ways, such as:

- Reference to existing phytosanitary requirements
- Indexed to estimated economic losses
- Expressed on a scale of risk tolerance
- Compared with the level of risk accepted by other countries.

The ISPM explains: “Overall risk is determined by the examination of the outputs of the assessments of the probability of introduction and the economic impact. If the risk is found to be unacceptable, then the first step in risk management is to identify possible phytosanitary measures that will reduce the risk to or below an acceptable level. Measures are not justified if the risk is already acceptable or must be accepted because it is not manageable (as may be the case with natural spread). Countries may decide that a low level of monitoring or audit is maintained to ensure that future changes in the pest risk are identified.”

The most restrictive phytosanitary measure is the imposition of a prohibition. The least restrictive phytosanitary measures would be in association with the importation of a commodity produced in a pest free area, pest free place of production, or pest free production site.

Other pest risk management measures that may be selected under a systems approach include pest freedom requirements for planting stock; pre-planting or pre-season, growing season, or post-harvest treatments; post-harvest handling, storage, and packaging requirements; geographic area or seasonal entry restrictions; post-entry inspection and laboratory testing requirements; and post-entry disposal and use restrictions.

3.1 Pest-Free Area

No phytosanitary requirements related to the pest in question, except origin certification, shall be imposed for potatoes moving from an officially recognized pest-free area. ISPM numbers 4, 8 and 10 should guide the pest-free area, pest free places of production and pest free production site determinations.

3.2 Systems Approaches

ISPM #14, The Use of Integrated Measures in a Systems Approach for Pest Risk Management defines “Systems Approach” as “The integration of different pest risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of phytosanitary protection”.

ISPM #14 explains that, “A systems approach integrates pest risk management measures to meet the appropriate level of phytosanitary protection of the importing country. Systems approaches provide, where appropriate an equivalent alternative to procedures such as disinfestation treatments or replace more restrictive measures like prohibition. This is achieved by considering the combined effect of different conditions and procedures. Systems approaches provide the opportunity to consider both pre- and post-harvest procedures that may contribute to the effective management of pest risk. It is important to consider systems approaches among risk management options because the integration of measures may be less trade restrictive than other risk management options (particularly where the alternative is prohibition).”

This standard recognizes approved, official seed potato certification programs as “Systems Approaches” to potato pest risk management. Other risk mitigation measures or methods, described in section 4 of this standard or in ISPM No. 14, where appropriate and justified, can be added to achieve any specified, acceptable level of risk for seed potatoes, tablestock, or potatoes for processing.

3.3 Seed Potato Certification

This standard assumes that all work plans for international seed potato trade will include only officially certified seed potatoes. Seed potato certification schemes represent systems approaches to pest risk management for plants for planting. Appendix 2 outlines criteria for seed potato certification.

While intensive and highly sensitive testing methods are required for microplantlets, microtubers, and minitubers; and for certain pathogens in field grown seed, certification of field grown seed potatoes relies primarily on visual inspection. In addition, seed potato certification programs include tolerances for potato pests. Consequently, seed potato certification alone may not result in an acceptable level of risk for quarantine pests.

Subsections 3.3.1 - 3.3.3 describe potato propagule risk management measures that are commonly integrated into current seed potato certification schemes. Appendix 2 describes other seed potato certification risk management measures.

- 3.3.1 Aseptic laboratory production of microplantlets and microtubers (nuclear stock) from parent material that is found to be free from all regulated pests. Appendix 4 specifies testing and other requirements.
- 3.3.2 Insect proof, greenhouse (or in other protected environment) production of minitubers from aseptic, laboratory produced, microplantlets or microtubers that complies with the requirements specified in Appendix 4.
- 3.3.3 Field produced tubers randomly sampled and visually inspected, tested in the laboratory or tested in a field test plot and found to be free from or within tolerance for regulated pests.

3.4 Preclearance

Preclearance may include verification of compliance with a mutually agreed upon work plan for potato trade between trading partners. The NAPPO Preclearance Standard (RSPM 2) provides additional details on preclearance.

3.5 Germplasm Introduction

- 3.5.1 Uncertain quarantine pest status: Post-entry quarantine is a means of allowing the entry, under permit, of potato germplasm of uncertain quarantine pest status. Germplasm from

wild, native and field grown origins must be held in tissue culture and tested as specified in Appendix 4.

3.5.2 Approved Pest-Free Places or Sites: Microplantlets, microtubers, or minitubers that have been produced in pest free places of production and production site approved by the NPPO and in compliance with ISPM 4 and/or 10 may be imported. When potato microplantlets, microtubers, or minitubers meet the requirements specified in Appendix 4, they may be imported, subjected to minimal sampling and audit testing and then distributed within the NAPPO region.

3.5.3 Propagule Testing: Germplasm of uncertain quarantine pest status, placed into tissue culture, must be tested for those pathogens not eliminated by the tissue culturing process, that is, quarantine viruses and bacteria prior to release to the industry. Acceptable test methods are given in Appendix 4. Microplantlets, microtubers, or minitubers imported from approved pest free places or sites of production do not require testing for all quarantine viruses and bacteria but an audit of them for freedom from pathogens is desirable. Audit testing may be limited to one or a few pathogens that are most likely to infect the microplantlets, microtubers, or minitubers and serve as surrogate(s) for other regulated and quarantine pathogens.

3.6 Research (Special Permit)

Even when potatoes are otherwise prohibited entry, at the discretion of the importing country's national plant protection organization, they may be permitted entry for research purposes. Research permits require that all handling and research be authorized by the NPPO, including use of a secure facility. Typically, destruction of the imported material and any progeny is required when the research is completed.

3.7 Prohibition

A prohibition is a total ban on the entry of potentially infested commodities. Prohibitions should be temporary and employed as emergency measures, during the time required to evaluate the pest risk and determine the availability of appropriate pest risk management measures.

3.8 Risk Management Measures

ISPM 14, "The Use of Integrated Measures in a Systems Approach for Pest Risk Management" lists many risk management measures. Subsections 4.1 - 4.3 list the measures that apply to potatoes.

3.8.1 Growing Season Measures

Cultural practices include the tissue culture production of microplantlets and microtubers, the greenhouse production of minitubers, crop rotation, flooding, production in artificial media, physical and/or geographic isolation, etc.

- Field inspection of the growing crop for regulated pests.
- Field sampling of the growing crop and laboratory diagnostics for regulated pests.
- Field treatments to control regulated pests.

3.8.2 Post-Harvest Measures

- Sampling of harvested/packed potatoes and inspection to determine the incidence of regulated pests.
- Sampling of harvested/packed potatoes and laboratory diagnostics to determine the incidence of regulated pests. Appendix 5 specifies pre-shipment testing requirements for PVY^N.
- Potato treatments to eliminate regulated pests include washing, chemical, heat and cold, irradiation, controlled atmosphere, etc.

- Treatment to inhibit the germination of fresh consumption or processing potatoes.
- Special packaging requirements may be designed to prevent subsequent pest infestation and prevent tampering that could void the protection achieved by growing season or post-harvest pest risk management measures.

3.8.3 Restrictions on End Use

ISPM 11, “Pest Risk Analysis for Quarantine Pests”, cites restrictions on end use, distribution and periods of entry of the commodity as options for risk management in consignments. Potatoes are used for several purposes including planting; fresh product consumption by the public or restaurants; and processing into frozen products, potato chips, and potato flakes and starch.

The end use of potatoes will affect the associated risk for the product. Restrictions for potatoes can, where appropriate and justified, be determined based on end use. An additional restriction, for example, on fresh market potatoes would be the application of a sprout inhibitor as a means of discouraging diversion for planting. Potatoes for processing could be restricted to certain locations where any risk associated with holding, handling, processing, and waste disposal can be managed effectively.

4. Potato Risk Management Standards

Subsections 4.1 - 4.3 briefly discuss risk management relative to potatoes and potentially associated pathogens, insects, and nematodes. Background information on pathogens and nematodes is provided in Appendix 3. Table I (by commodity) and Table II (by pest category) graphically display the pest risk management measures in this standard.

Table stock potatoes and potatoes for processing present less risk for many potato pests. Consequently, restrictions placed on the movement of table stock potatoes and potatoes for processing may be less than those for seed potatoes.

4.1 Pathogens

In this standard, "pathogens" includes viruses, viroids, phytoplasmas, bacteria and fungi. In potatoes, these agents can cause a number of diseases that result in varying degrees of economic impact ranging from delayed maturity and increased agricultural inputs to reductions in yield, storage tuber quality and viability losses.

When potato microplantlets, microtubers, and minitubers are moved into the NAPPO countries, risk management measures specified in Appendix 4 must be applied to reduce the risk of introduction of pathogens. Other risk management measures may be applied to reach the appropriate level of protection for the importing country.

4.2 Insects

While the tuber moth, wireworms, flea beetles, etc. can be tuber-borne, the NAPPO regulated insect pests are highly unlikely to be moved in graded seed, fresh market, or processing potatoes. Unwashed bulk, un-graded (field run) tubers contaminated with potato haulms could carry these pests and present a greater risk. Fresh market and potatoes for processing should be graded, washed, and packed in a manner that prevents the movement of insect pests.

When potato propagules are moved into and among NAPPO countries, risk management measures specified in Appendix 4 must be applied to reduce risk of introduction of insect

pests. Other risk management measures may be applied to reach the appropriate level of protection for the importing country.

4.3 Nematodes

Plant parasitic nematodes fall into three main categories: ectoparasitic, migratory endoparasitic, and endoparasitic. Plants or tubers with soil present the greatest risk of pest introduction. Additional information on nematodes may be found in Appendix 3, “Background Information on Pathogens and Nematodes”.

Nematode-free plants grown in aseptic culture or in non-soil or treated soil media that is protected against nematode infestation present no significant pest risk. When potato propagules are moved into NAPPO member countries, risk management measures specified in Appendix 4 must be applied to reduce the risk of introduction of nematode pests. Other risk management measures may be applied to reach the appropriate level of protection for the importing country. Pest risk can be managed by the following:

- Planting nematode free seed in nematode free areas.
- Planting nematode-free (certified) seed potatoes in soil treated to eliminate nematodes during the growing season.
- Planting nematode-free (certified) seed potatoes combined with sampling, handling, nematode extraction, and laboratory analysis to determine whether nematodes are present.

5. Documentation Standards

5.1 Phytosanitary Certificate

When required by the importing country, a Phytosanitary Certificate based on the IPPC model certificates must be issued by an authorized certification official of the exporting country to certify compliance with the phytosanitary legislation and phytosanitary regulations of the importing country.

5.2 Seed Potato Certification

Tags, seals or other indicia of seed potato certification must be affixed to bags, cartons, bins or conveyances to certify that the requirements of an official seed potato certification scheme have been met.

6. Notification

As noted in the Introduction of this standard, several International Standards for Phytosanitary Measures (ISPMs) apply, in whole or part, to this standard. Two ISPMs relevant to the subject of notification are 13 and 17 that NAPPO member countries should follow when applying this standard.

ISPM 13, “Guidelines for the Notification of Non-Compliance and Emergency Action.”

This standard describes the actions to be taken by all countries regarding the notification of:

- A significant instance of failure of an imported consignment to comply with specified phytosanitary requirements, including the detection of specified regulated pests.
- A significant instance of failure of an imported consignment to comply with documentary requirements for phytosanitary certification.
- An emergency action taken on the detection in an imported consignment of a regulated pest not listed as being associated with the commodity from the exporting country.

- An emergency action taken on the detection in an imported consignment of organisms posing a potential phytosanitary threat.

ISPM 17, “Pest Reporting”. This standard describes the responsibilities of and requirements for contracting parties in reporting the occurrence, outbreak and spread of pests in areas for which they are responsible. It also provides guidance on reporting successful eradication of pests and establishment of Pest Free Areas.

Table 1: Options for Phytosanitary Measures by Category of Potato Product

COMMODITY	PEST FREE AREAS ¹	SEED POTATO CERTIFICATION	GROWING SEASON				POST-HARVEST					PRECLEARANCE/ ORIGIN INSPECTION	POST-ENTRY QUARANTINE	RESEARCH PERMIT	EMERGENCY, TEMPORARY PROHIBITION	
			C	I	D	T	I	D	T	G	P					
Microplantlets	Y	Y	Y	-	Y	Y	-	Y	-	-	Y	Y	Y	Y	Y	Y
Microtubers	Y	Y	Y	-	Y	Y	-	Y	-	-	Y	Y	Y	Y	Y	Y
Cuttings	Y	Y	Y	Y	Y	Y	Y	Y	-	-	Y	Y	Y	Y	Y	Y
Minitubers	Y	Y	Y	Y	Y	Y	Y	Y	-	-	Y	Y	Y	Y	Y	Y
Seed Tubers	Y	Y	Y	Y	Y	Y	Y	Y	S	-	Y	Y	Y	Y	Y	Y
Table Stock	Y	S	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N/A	N/A	Y
Processing Potatoes	Y	S	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N/A	N/A	Y
True Seed	Y	Y	Y	Y	-	-	Y	-	-	-	Y	Y	Y	Y	Y	Y

¹Includes pest free areas, pest free places of production, and pest free production sites.

Legend:

- C** = Cultural practices
- I** = Inspection
- D** = Laboratory diagnostics
- T** = Treatments
- G** = Germination inhibition
- P** = Packaging requirements
- S** = This risk management measure could apply to some pests.
- Y** = Signifies that the measure applies.
- = The risk management measure is not appropriate or practical.

Table 2: Options for Phytosanitary Measures by Pest

PEST CATEGORY	PEST FREE AREAS ¹	SEED POTATO CERTIFICATION	GROWING SEASON		POST-HARVEST							PRECLEARANCE/ ORIGIN INSPECTION	POST-ENTRY QUARANTINE	RESEARCH PERMIT	EMERGENCY/TEMPORARY PROHIBITION
			C	I	D	T	I	D	T	G	P				
Viruses	Y	Y	Y	Y	Y	-	-	Y	-	Y	-	Y	Y	Y	Y
Viroids	Y	Y	Y	Y	Y	-	-	Y	-	Y	-	Y	Y	Y	Y
Phytoplasmas	Y	Y	Y	Y	Y	-	-	Y	-	Y	-	Y	Y	Y	Y
Bacteria	Y	Y	Y	Y	Y	-	S	Y	-	Y	-	Y	Y	Y	Y
Fungi	Y	Y	Y	Y	Y	-	S	Y	Y	Y	-	Y	Y	Y	Y
Insects	Y	Y	Y	Y	Y	Y	S	Y	Y	Y	Y	Y	Y	Y	Y
Ectoparasitic Nematodes	Y	Y	Y	-	Y	-	S	Y	Y	Y	Y	Y	Y	Y	Y
Endoparasitic and, Migratory endoparasitic nematodes	Y	Y	Y	-	Y	-	S	Y	-	Y	-	Y	Y	Y	Y

¹Includes pest free areas, pest free places of production, and pest free production sites.

Legend:

- C** = Cultural practices
- I** = Inspection
- D** = Laboratory diagnostics
- T** = Treatments
- G** = Germination Inhibition
- P** = Packaging requirements
- S** = This risk management measure could apply to some pests.
- Y** = Signifies that the measure applies.
- = The risk management measure is not appropriate or practical

Appendix 1

Regulated Pests for NAPPO Countries

List 1: Quarantine Pests for NAPPO countries

Scientific name	Common name	Canada	Mexico	USA
Viruses				
Andean potato latent virus (APLV)		QA	QA	QA
<i>Andean potato mottle virus</i> (APMoV)		QA	QA	QA
Arracacha virus B - oca strain (AVB-O)		QA	QA	QA
<i>Beet curly top virus</i> (BCTV)		QA	QA	QP
Potato deforming mosaic virus (Brazil) (PDMV)		QA	QA	QA
<i>Potato virus T</i> (PVT)		QA	QA	QA
<i>Potato virus U</i> (PVU)		QA	QA	QA
<i>Potato virus V</i> (PVV)		QA	QA	QA
<i>Potato virus Y</i> , strain Yc (PVYc)		QA	QA	QA
Potato yellow vein virus (PYVV)		QA	QA	QA
Potato yellowing virus (PYV)		QA	NR	QA
Solanum apical leaf curling (SALCV)		NR	NR	QA
Tobacco ringspot virus - calico strain (TRSV-Ca), a		QA	QA	QA
<i>Tobacco streak virus</i> , potato strain (TSV)		NR	QA	NR
<i>Tomato spotted wilt virus</i> (TSWV)		NR	QA	NR
Viroids				
<i>Potato spindle tuber viroid</i> (PSTVd)		QP	QA	QP
Bacteria				
<i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i>	Bacterial ring rot	RNQ	QA	RNQ
<i>Erwinia chrysanthemi</i> (potato strain)	Soft rot	RNQ	QA	RNQ
<i>Ralstonia solanacearum</i> race 3	Brown rot	QA	QA	QA
Fungi				
<i>Angiosorus (Thecaphora) solani</i>	Smut	QA	QA	QA
<i>Oospora pustulans</i> (syn. <i>Polyscytalum pustulans</i>)	Skin spot	RNQ	QA	RNQ
<i>Puccinia pittieriana</i> P. Henn	Common rust	QA	NR	QA
<i>Synchytrium endobioticum</i> (Schilbersky) Percival	Potato wart	QP	QA	QA
Nematodes				
<i>Ditylenchus destructor</i> Thorne	Potato rot	QP	QA	QP
<i>Ditylenchus dipsaci</i> (potato and maize races)	Stem and bulb	QA	QA	RNQ
<i>Globodera pallida</i> (Stone) Behrens	Pale cyst	QP	QA	QA
<i>Globodera rostochiensis</i> (Wollenweber) Behrens	Golden	QP	QP	QP
<i>Meloidogyne chitwoodi</i> (Golden et al, 1980)	Columbia root knot	QA	QP	RNQ
Insects				
<i>Premnotrypes</i> spp.	Andean root weevil	NR	QA	NR
<i>Leptinotarsa Decemlineata</i>	Colorado potato beetle	QP	QA	NR

NR: Not regulated

QA: Quarantine pest, not present (absent) in an area (ISPM 19).

QP: Quarantine pest, present but not widely distributed and under official control (ISPM 19)

QT: Quarantine pest, transient

RNQ: Regulated Non-Quarantine pest

List 2: Regulated Non-Quarantine Pests for NAPPO Countries

Scientific name	Common name	Canada	Mexico	USA
Viruses				
Potato latent virus (PotLV)	Red la Soda Virus	RNQ	NR	RNQ
<i>Potato leafroll virus</i> (PLRV)		RNQ	RNQ	RNQ
<i>Potato virus A</i> (PVA)		RNQ	RNQ	RNQ
<i>Potato virus M</i> (PVM)		RNQ	NR	RNQ
<i>Potato virus S</i> (PVS)		RNQ	RNQ	RNQ
<i>Potato virus X</i> (PVX)		RNQ	RNQ	RNQ
<i>Potato virus Y</i> , strain Yo (PVYo)		RNQ	RNQ	RNQ
<i>Tobacco rattle virus</i> (TRV)		RNQ	QA	RNQ
Bacteria				
<i>Streptomyces scabies</i>	Common scab	RNQ	RNQ	RNQ
<i>Erwinia carotovora</i> subsp. atroseptica	Blackleg	RNQ	RNQ	RNQ
<i>Erwinia carotovora</i> subsp. carotovora	Soft rot	RNQ	RNQ	RNQ
Fungi				
<i>Fusarium</i> spp.		RNQ	RNQ	RNQ
<i>Helminthosporium solani</i>	Silver scurf	RNQ	NR	RNQ
<i>Phytophthora infestans</i>	Late blight	RNQ	RNQ	RNQ
<i>Rhizoctonia solani</i>	Rhizoctonia; black scurf	RNQ	RNQ	RNQ
<i>Spongospora subterranea</i>	Powdery scab	RNQ	RNQ	RNQ
<i>Verticillium albo-atrum</i>	Wilt	RNQ	RNQ	RNQ
<i>Verticillium dahliae</i>	Wilt	RNQ	RNQ	RNQ
Insects				
<i>Phthorimaea operculella</i>	Potato tuberworm	NR	RNQ	NR
<i>Epicaerus cognatus</i>	Potato weevil	NR	RNQ	QA

NR: Not regulated

QA: Quarantine pest, not present (absent) in an area (ISPM 19).

QP: Quarantine pest, present but not widely distributed and under official control (ISPM 19)

QT: Quarantine pest, transient

PFA: Pest Free Areas exist

RNQ: Regulated Non-Quarantine pest

List 3: Other Regulated Pests recommended for further evaluation by NAPPO countries.

Scientific name	Common name	Canada	Mexico	USA
Viruses				
Dulcamara mottle (Genus Tymovirus)*		NR	NR	QA
Eggplant mottled dwarf virus (EMDV)*		NR	NR	QA
Pea streak virus (Alfalfa enation strain)*		NR	QA	NR
Potato mop top virus (PMTV)		RNQ	RNQ	QP
Potato virus Y, strain Yn group (PVYn/ntn)		QP	QT	QP
Potato yellow dwarf virus (PYDV)**		NR	QA	NR
Potato yellow mosaic virus (PYMV)*		NR	NR	QA
Tobacco necrosis virus (TNV)*		NR	QA	QA
Tomato black ring virus (TBRV)**		QA	QA	QA
Wild potato mosaic virus (WPMV)*		NR	NR	QA
Phytoplasma				
Potato marginal flavescescens**		NR	NR	QA
Potato parastolbur**		NR	NR	QA
Potato purple top wilt**		NR	NR	QP
Potato stolbur**		NR	QA	QA
Potato witches broom**		NR	QA	QP
Bacteria				
Potato leaflet stunt agent*		NR	NR	QA
<i>Streptomyces caviscabies</i> *	Common scab	NR	NR	QA
<i>Streptomyces turgidiscabies</i> *	Common scab	NR	NR	QA
Fungi				
<i>Aecidium cantensis</i>	Deforming rust	NR	NR	QA
<i>Cercospora solanicola</i> Atk.*		NR	NR	QA
<i>Cercospora solani - tuberosi</i> Thirumalachar*		NR	NR	QA
<i>Helicobasidium brebissonii</i> **		NR	QA	NR
<i>Ozonium texanum</i> (Texas root rot)*		NR	NR	QP
<i>Phoma andina</i> *	Phoma leaf spot	NR	NR	QA
<i>Phoma exigua</i> var. <i>foveata</i> (Foister) Boerema*	Gangrene	QA	NR	NR
<i>Polysaccopsis hieronymi</i> (Schract) P. Henn*		NR	NR	QA
<i>Verticillium nubilim</i> Pethybr.*	Twisted sprout of potato	NR	NR	QA
Nematodes				
<i>Heterodera glycines</i> (Ichinohe)*	Soybean cyst	QP	NR	NR
<i>Longidorus leptcephalus</i> Hooper*		NR	NR	QA
<i>Meloidogyne javanica</i> *		NR	QA	NR
<i>Meloidogyne acronea</i> Coetzee*		NR	QA	NR
<i>Meloidogyne exigua</i> **		NR	QA	NR
<i>Thecavermiculatus andinus</i> Golden et al*		NR	NR	QA
<i>Zygotylenchus guevarai</i> (Tobar Jimenex)		NR	NR	QA
Insects				
<i>Rhigopsidis tucumanus</i> *		NR	QA	NR
<i>Thrips angusticeps</i> *	Field thrips	NR	QA	NR
<i>Tipula paludosa</i> *		NR	QA	NR

*The NAPPO Potato Panel has recommended that the NAPPO member countries that have put these pests on their quarantine pest list should delete them or perform a pest risk analysis to justify them.

**The NAPPO Potato Panel has recommended that these pests be deleted from all NAPPO member country quarantine lists.

NR: Not regulated

QA: Quarantine pest, not present (absent) in an area (ISPM 19).

QP: Quarantine pest, present but not widely distributed and under official control (ISPM 19)

QT: Quarantine pest, transient

PFA: Pest Free Areas exist

RNQ: Regulated Non-Quarantine pest

Appendix 2

Criteria for Seed Potato Certification for NAPPO Countries

1. Roles

Historically, seed potato certification has been a regulatory pest control strategy that incorporates cultural practices. Requirements include guidelines for containers, grades, identification, inspection, isolation, maintenance, propagative material, record-keeping, sanitation, testing, and treatment. Tolerances are set to ensure that the potatoes meet specified quality requirements. Seed potato certification regulations provide quality assurance for vegetatively propagated planting stocks. Quality control requirements incorporated into seed potato certification regulations have been selected at the discretion of the national, state or provincial seed potato certification agency to satisfy industry needs for planting materials.

An increasingly important role for seed potato certification is the management of potato quarantine pest risk. To determine to what extent a particular seed potato certification regulatory scheme or system can serve as a valid and reliable potato quarantine pest risk management strategy, the importing country will need to evaluate both the administrative competency of the certification agency and the certification scheme.

It is important to note that seed potato certification program evaluation is only required when a particular program is being considered as a basis for the phytosanitary certification of seed potatoes. The criteria that are elaborated in this appendix in no way constitute a directive for the harmonization of the various seed potato certification schemes or systems that are employed nationally or locally in North America to provide quality assurance for potato planting materials. The criteria in this appendix could be used for determining what changes might be appropriate to facilitate foreign trade and to make seed potato certification programs equivalent.

2. Evaluation and Accreditation Criteria

2.1 Administrative Competency

Competent agencies:

- 2.1.1 Will be legally constituted under federal, state, or provincial law (this includes the naming of the certification agency as an official certification agency and the granting of the authority required to perform the full range of work necessary for valid and reliable certification).
- 2.1.2 Will employ inspection and laboratory diagnostic personnel that have the education, training, and experience necessary for detection and identification of pests and diseases; the proper collection, handling, and submission of samples for laboratory diagnostics; and recognition of potato varieties.

In addition, they must possess knowledge of a) all relevant laws and regulations, b) forms and their proper use, and c) identifying markings for certification and their proper use. The certification agency may accredit or approve university or private laboratories to perform valid and reliable diagnostics. See NAPPO Regional Standard for Phytosanitary Measures (RSPM) 9, “Accreditation of Laboratories”.

2.1.3 May recover the costs of the services they render; but, they should not profit from the sale of seed potatoes or have any other financial interests in their production or marketing. It is essential that the agency function as an impartial third party.

3. Certification System

Certification schemes should be limited generation systems. Clonal selection systems that involve continuous reproduction in the field are not limited generation systems. Limited generation system means a scheme wherein the number of increases made in the field is limited.

In North America, *in-vitro* products (pathogen-tested plantlets and microtubers) of aseptic laboratory culture should be identified as “pre-nuclear”. Progeny of pre-nuclear materials such as cuttings, plants, and minitubers produced in greenhouses, screenhouses or other controlled environments from pathogen-tested plantlets or microtubers in non-soil media or heat-treated soil (aerated steam at 140° F for 30 minutes or equivalent) should be identified as “nuclear”. See Appendix 5, “NAPPO Requirements for Potato Propagules” for additional relevant information.

The number of field grown generations between nuclear stock and the generation that is not eligible for seed certification should not exceed five, for purposes of international trade. It is strongly recommended that field progeny generation classes be identified simply as “Generation I”, “Generation II”, “Generation III”, “Generation IV”, and “Generation V”.

The following table shows the equivalency of terms used among the NAPPO member country certification agencies for seed potatoes harvested from a series of successive field plantings.

Limited Generation Seed Potato Field Planting Comparison Table

Country	YEAR IN THE FIELD						
	1 ¹	2	3	4	5	6	7
Mexico	B ²	R1	R2	R3	C	~ ~ ~	~ ~ ~
Canada	PE	E1	E2	E3	E4	F	C
United States	G1 ³	G2	G3	G4	G5	~ ~ ~	~ ~ ~

- Certification regulations for Canada and Mexico specify the pest and disease tolerances and other requirements for each field generation.
- The U. S. Export Standards for Seed Potatoes are contained in Appendix P of the United States Department of Agriculture Export Certification Manual. Specific pest and disease tolerances and other requirements for each field generation are specified.

¹The first field planting utilizes laboratory-tested stocks, which may be tissue-cultured plantlets, greenhouse-produced minitubers, stem cuttings, or line selections. Contact agencies for details as to types of stocks planted in their programs.

²Terms used for seed potatoes for a particular year in the field: B=basic, C=certified, E=elite, F=foundation, G=generation, N=nuclear, PE=pre-elite, R=registered.

³Based on the U. S. Export Standards for Seed Potatoes. Some states certify additional generations. These additional generations are intended for domestic interstate and intrastate use.

3.1 Certification Program Elements

Seed potato certification schemes will address the following:

- 3.1.1 Application for certification: The form, content and time requirements for applications for certification enables the certification agency to a) determine the eligibility of the propagative material described by the applicant, b) locate and approve each field entered for certification, c) trace the lineage of all stock entered for certification back to its origin as an acceptable generation, as nuclear, or as pre-nuclear stock, and d) complete and maintain all paperwork necessary for documenting required inspection, testing, and other certification requirements.
- 3.1.2 Definitions: All terms used in the certification program should be defined in the certifying agency's regulations governing certification of seed potatoes. This helps to ensure a clear understanding of the agency's requirements.
- 3.1.3 Diagnostics: "Diagnostics" cover all official functions and activities performed by or under the direction of qualified scientists-usually in a laboratory setting. "Diagnostics" include but are not limited to 1) sample processing for the recovery or isolation and identification of pathogens, insects, nematodes and other pests; 2) pest identification utilizing morphological characters such as for insects, mites, and other arthropods and nematodes; 3) disease determinations utilizing indicator plants; 4) serological tests such as Enzyme Linked Immuno-Sorbent Assays (ELISA); and 5) assays based on nucleic acid amplification by various Polymerase Chain Reaction (PCR) methodologies.

Selection of the diagnostics required for seed certification is at the discretion of the seed potato certification agency. Diagnostics required to meet the regulated pest phytosanitary requirements of a trading partner might be different than those included in the agency's regulations and may need to be negotiated between the National Plant Protection Organizations (NPPO) of the importing and exporting countries.

- 3.1.4 Eligibility: For each class of seed that is to be certified, seed potato certification agencies should prescribe the requirements that propagative material must meet. In some cases, this could include pre-season field plot testing.

Agencies should prescribe the pest freedom requirements (pest and disease tolerances) that must be met at the time of inspection. Agencies may require post-season (post-harvest) field plot testing as a condition of eligibility for certification during the next growing season.

Agencies may require that, in order to be eligible for participation in the program, growers not produce table stock at all, or not grow table stock in the same vicinity or on the same farming operation or using the same equipment.

- 3.1.5 Sanitation: Certain potato pathogens can be spread through the use of contaminated equipment, storage facilities, and containers. Notable examples are spindle tuber viroid and bacterial ring rot. Therefore, the seed potato certification agency's regulations should prescribe cleaning and treatment procedures that it deems to be necessary to decontaminate articles capable of spreading pathogens or pests. Compliance with prescribed sanitation requirements will be the responsibility of the seed potato grower. This, together with procedures for assuring compliance and grower accountability, should be specified in the seed potato certification agency's regulations.

- 3.1.6 Isolation: Certain potato pests can be spread by: wind-borne aerosols and infested soil, the movement of contaminated water, and the movement and feeding activity of viruliferous insects. Consistent with pest freedom standards prescribed by the seed potato certification agency, geographical or physical isolation requirements are utilized to limit such spread.

The aseptic laboratory production of pre-nuclear plantlets and microtubers from propagative stock that has been freed of pests is representative of the most stringent of the isolation strategies. Physical isolation for the production of nuclear stock (cuttings, plants, and minitubers) is achieved by growing pre-nuclear stock in non-soil media or heat-treated soil (aerated steam at 140° F for 30 minutes, or equivalent) inside insect-proof greenhouses, screenhouses, or other controlled environment facilities. Aseptic laboratories and insect-proof greenhouses might qualify as pest free production sites.

To avoid the spread of pests to seed potatoes being produced in the field, certification agencies should prescribe various distances to separate seed fields from other fields where potatoes or other hosts of potato pests are being grown. To satisfy its own national, state, provincial, or local seed quality needs, the geographic isolation requirements are at the discretion of the seed potato certification agency. As in the case of diagnostics, geographic isolation requirements to provide quarantine pest security may be negotiated between the trading partners and specified in a bilateral work plan.

- 3.1.7 Maintenance: The visual detection of potato pests and diseases during the performance of growing season field inspections by the seed potato certification agency's field personnel depends heavily, among other things, on plant vigor and disease expression. Disease symptoms can be masked by low, as well as too much, plant vigor.

Diseased plant visibility can be obscured or blocked by weeds growing in the field. Therefore, potato certification agencies should require the grower to maintain seed potato plantings in good growing condition and provide that certification may be refused when weediness and other field conditions prevent adequate or proper inspection.

Control for pests that can serve as vectors of pathogens also may be prescribed. Consistent with this fact, the presence of disease vectors may be a cause for refusal of certification.

- 3.1.8 Inspection: The seed potato certification agency's regulation should prescribe a minimum of two field inspections during the growing season. The first inspection usually is performed, before the rows close, in order to detect the presence of plant diseases, off-type plants and varietal mixtures.

The second field inspection is performed when the age of the plants is such that the symptoms of potato diseases caused by bacteria (blackleg and bacterial ring rot are examples) and fungi (wilts are examples) can be detected. Certain varietal mixtures and diseases can also be evident at the time of the second field inspection.

Seed potato certification agencies may prescribe additional inspections to be performed during the growing season and at the time of harvest, while tubers are in storage, or at the time of grading (sorting) and packing. Additional such inspections focus on 1) tubers for the detection of rots or symptoms of nematode infestation, 2) yields, 3) varietal mixtures, 4) storage conditions, or 5) compliance with the agency's prescribed grade requirements.

3.1.9 Pre-plant testing: A seed potato certification agency may require pre-season sampling and field plot testing as a condition of potato seed lot eligibility for certification. The purpose is to 1) ensure proper identification of the seed lots that are to be planted, 2) help prevent substitution of seed lots, 3) exclude lots that do not meet eligibility requirements, and 4) facilitate inspection of fields planted using the seed lot and entered for certification by the agency.

3.1.10 Post-harvest testing: Many seed potato certification agencies require post-harvest sampling and greenhouse, field plot or laboratory testing of seed lots that have passed all growing season inspection requirements and which the grower intends to enter for certification during the next growing season or to sell to another seed potato grower for certification during the next growing season.

The purposes are to detect and exclude any seed lot 1) into or within which there has been disease spread after the time of the last growing season inspection, and 2) into which tubers of another variety might have been introduced during the harvest, handling and storage processes.

3.1.11 Tolerances: Pest freedom, varietal mixture, and other tolerances must be clearly specified in the seed potato certification agency's regulations. This is important to enable evaluation of the degree to which the certification scheme or strategy reduces pest risk to an acceptable level.

3.1.12 Classes: Seed potato classes are categories of quality. Classes represent levels of varietal purity and freedom from specific potato pests and diseases, consistent with specified tolerances.

3.1.13 Tuber Grades/Standards: Grades generally specify tuber size and shape; freedom from injury; decay; and freedom from tuber-borne pathogens, pest arthropods, and root knot (*Meloidogyne* spp.) nematodes.

3.1.14 Certification: Seed potato certification agencies must specify the requirements or conditions for certification and the bases for rejection.

3.1.15 Identification: Tags, seals, and certificates are among the various indicia of certification that can be used to properly identify potatoes that have been certified officially. Typically, tags and seals are affixed to containers whereas certificates accompany shipping manifests.

Certification tags and certificates minimally should enable the buyer, another certification agency, or a plant protection agency to identify the certifying agency, grower, seed lot, class and the grade, and variety of the seed in the container.

Certification tag colors are used to identify class or grade. The seed potato certification agency's regulations should specify whether and how tag colors are to be used in its identification scheme.

3.1.16 Record-keeping: Certification agencies must develop and utilize forms or other suitable methods for the capture and maintenance of data relating to each aspect of its certification scheme. Minimally, this should include data that would enable the trace-back of the propagative stock to its field generation, nuclear, or pre-nuclear origin; identification of the seed potato grower(s) and certification agency(s); inspections and tests performed; etc. Growers should also maintain records to verify 1) their seed purchases and sales, and 2) all activities for which they are responsible and accountable such as cleaning and sanitation,

cultural practices, pesticide applications, etc.

Diagnostic procedures and methods must be generally accepted as valid and reliable. Reports in refereed scientific journals that provide data showing validity and reliability constitute one basis for determining compliance with this requirement.

A government seed potato certification agency generally will employ its own laboratory to perform certification diagnostics. Such government diagnostic laboratories need not be formally accredited. They should, however meet the same standards as any other diagnostic laboratory that might be accredited or approved to perform diagnostics for certification purposes. NAPPO RSPM 9, “Accreditation of Laboratories” should be used as a guide whenever official laboratory accreditation is performed.

Appendix 3

Background Information on Pathogens and Nematodes

1. Pathogens

Many pathogens are tuber-borne. The correlation however between infected tubers prior to planting and diseased plants in the field is high with viroids and most viruses, and low with phytoplasmas. Bacterial and fungal pathogens that cause early vine death and tuber rots fall in the middle range of correlation.

The poor correlation for the phytoplasmas is because many infected tubers do not germinate or germinate very slowly and get excluded from diseased plant counts. These small plants typically produce tubers that are too small to be harvested or packed as part of any seed or fresh market product.

Environmental conditions often have a major impact on the correlation between bacterial and fungal infected tubers in an infested seed lot and diseased plants in a field planted from the seed lot. This is especially notable with the *Erwinia* species that cause the soft rot and blackleg diseases of stems and tubers.

Alone, few of the diseases caused by these pathogens cause major economic losses in potatoes until the percentage of diseased plants in a field becomes significant. The notable exception is bacterial ring rot, which must be kept very low in order to prevent significant economic losses in the commercial crops produced by using infected seed lots.

2. Nematodes

Since soil can easily transmit all three categories of nematode pests, tubers contaminated with any significant amount of adhering, infested soil would present a pest risk. Washed potatoes intended for the fresh or fresh processing market that are essentially free from soil present little risk for regulated ectoparasitic and cyst nematodes, including cyst nematodes such as *Globodera rostochiensis* and *Globodera pallida*.

Washing does not reduce the nematode pest risk associated with potato plants or seed tubers that are infested with endoparasitic or migratory endoparasitic nematodes, such as *Ditylenchus destructor* and species of *Meloidogyne*. The only effective risk management strategies are:

- 2.1 Production of seed potatoes in pest-free areas, pest free production sites or pest free places of production; or
- 2.2 Planting nematode-free (certified) seed potatoes in soil treated to eliminate nematodes; or
- 2.3 Planting nematode-free (certified) seed potatoes combined with application of other measures: sampling, handling, nematode extraction, and laboratory analysis during the growing season to determine whether nematodes are present.

Pest risk also is associated with the end use of potatoes and the colonization potential of a particular species. Fresh market potatoes and fresh potatoes for processing typically are not planted and the waste products that result from food preparation and commercial processing generally are disposed of in ways and at locations that reduce the probability of colonization and establishment to an acceptable level. Therefore, fresh market and fresh potatoes for processing, treated to inhibit germination and consequently to deter their use for planting have reduced pest risk. Unwashed bulk or field run potatoes typically are contaminated with soil and would present significant, unreduced risks.

Appendix 4

Requirements for Potato Germplasm and Minitubers for NAPPO Countries

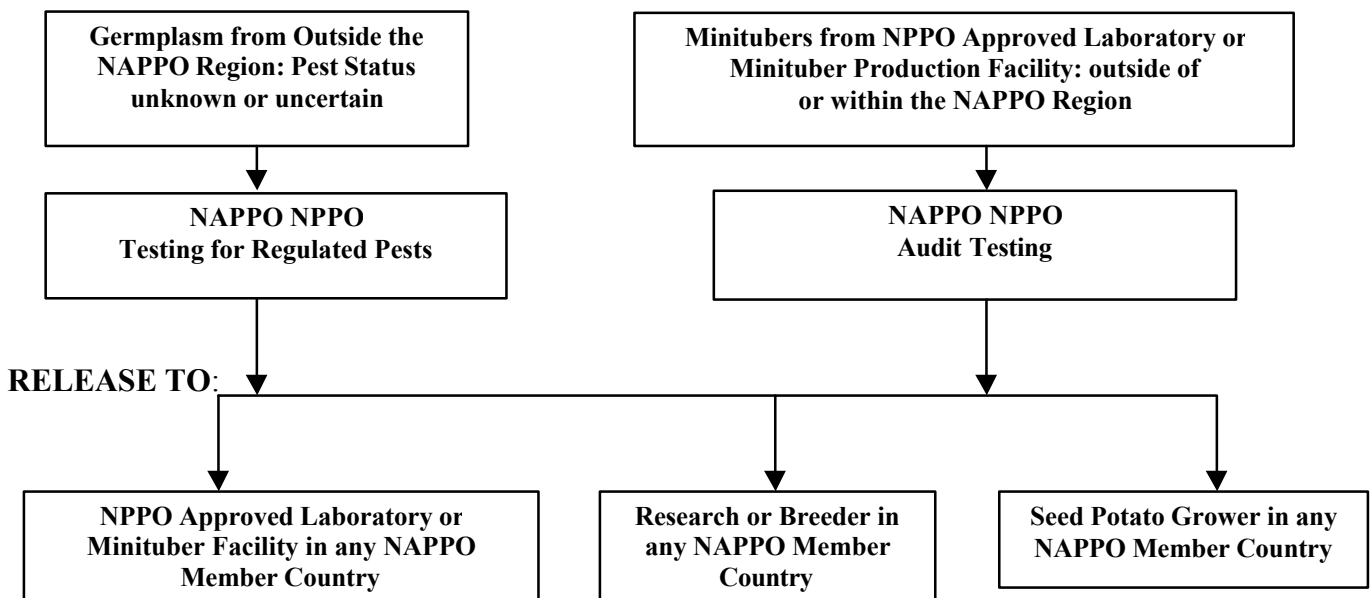
1. Introduction

This appendix specifies phytosanitary actions for managing the pest risk associated with the movement of germplasm of tuber-bearing *Solanum* species (potato) into the NAPPO region and commercial trade of minitubers into and within the NAPPO region (although minitubers are the usual commercial commodity produced in a protected environment, commercial trade of microtubers or micropropagated plantlets are to be considered equivalent to minitubers for trade purposes). The specified phytosanitary actions vary both with the source of the potato plants or plant parts and their end use. The introduction of potato germplasm and minitubers may be desirable for either of two general uses:

- Small quantities for research, including use in breeding programs or
- Large numbers for commercial cultivation.

The objective of this appendix is to: describe the phytosanitary requirements that potato propagules must meet to be imported or moved into NAPPO countries.

The pest risk associated with the introduction of potato germplasm will be reduced to an acceptable level when each NAPPO member country properly applies the phytosanitary actions specified in this appendix. Consequently, germplasm officially tested by a NAPPO national plant protection organization (NPPO) or approved laboratory may be released for movement into and among the NAPPO member countries. The following schematic displays how such movement may occur.



2. Phytosanitary Actions for Potato Germplasm

2.1 Basic Phytosanitary Considerations

Whereas the risk associated with introduction of wild, native and field grown potato germplasm usually is high, the risk is low relative to the importation of minitubers that are freed of and continually monitored for plant pathogens and micropropagated *in vitro* in laboratories under aseptic conditions.

Specified phytosanitary actions take into account the fact that many pests and pathogens can be eliminated by aseptic micropropagation and obligate endophytic pathogens can be detected using detection or diagnostic methods and eliminated using therapeutic methods.

2.2 Basic Phytosanitary Actions

NAPPO member country must ensure that all imported potato germplasm is:

- Received *in vitro* or placed into tissue culture by a NPPO approved laboratory,
- Tested for all the regulated pests cited in this appendix (Table 1), and
- Diagnostic and other records are kept to enable trace-back.

Potato germplasm received as *in vitro* microplantlets must be visually inspected to ensure they are free from microbial contaminants and that the sterile condition was maintained during the shipping process. To enhance the effectiveness of the inspection process, microplantlets should be transferred into an enrichment medium and held prior to inspection, long enough for microbial development to occur. Inspection or testing for nematode and insect pests is not required, since they are eliminated by aseptic tissue culturing of germplasm.

All tests for potato pathogens are to be conducted by or under the supervision of a plant pathologist employed or approved by the NAPPO member country's national plant protection organization (NPPO) or in a laboratory that meets the criteria in the NAPPO RSPM 9, "Accreditation of Laboratories".

Accuracy of laboratory tests depends on sample quality and test methodology, both of which should be identified in Standard Operating Protocols. The result of a test is valid only if it is carried out correctly by a skilled analyst. In some cases a confirmatory test is required to ensure adequate reliability of the result. Acceptable tests identified in this Appendix provide a guide to available tests but new and other validated methodologies may also be adopted to provide an accurate determination for the presence or absence of a pathogen.

Potato germplasm tested and not found to be free from regulated pests must be "cleaned-up" before release or destroyed in a manner that precludes escape of any quarantine pathogen into the environment.

2.3 Specific Phytosanitary Actions

Germplasm from Wild, Native and Field Grown Origins: Potato germplasm from wild and native and from field-grown origins outside of the NAPPO region must be put into tissue culture upon receipt by the importing NAPPO member country and tested for all the regulated pests cited in this appendix. (Table 1).

2.3.1 Acceptable Tests for Regulated Viruses/Viroids

2.3.1.1 Viruses/Electron Microscopy: Electron microscopy can serve as a useful initial tool followed by more specific test methodologies to determine whether regulated viruses are present. Identification of the specific virus might not be possible, since the virus particles of some non-quarantine viruses can appear to be similar to those of quarantine virus(es) of concern.

2.3.1.2 Viruses/Serological and Molecular Methods: Regulated viruses include potato viruses A (PVA), M (PVM), S (PVS), X (PVX), T (PVT), U (PVU), and Y (PVY), potato latent virus (PotLV), and potato leaf roll virus (PLRV), Potato mop-top virus (PMTV), and tobacco rattle virus (TRV). ELISA tests, using commercially available antibodies or kits, can be used to detect most of these viruses. Molecular tests such as RT-PCR and dot blot hybridization with appropriate primers and probes are also acceptable. Except for PLRV, these viruses also can be rapidly detected by electron microscopic examination of negatively stained crude sap preparations.

Tests for quarantine viruses must be performed on tissue samples from at least 4-6 week old plants, which are at least 15 cm tall and grown in a pest-free medium.

2.3.1.3 Viruses/Biological Assays: Mechanically transmitted regulated viruses can be detected by inoculation to indicator hosts such as: *Capsicum annuum*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Lycopersicon esculentum*, *Nicotiana benthamiana*, *N. bigelovii*, *N. clevelandii*, *N. debneyi*, and *N. tabacum* cv 'White Burley' and 'Samsom' (Table 2). Visual inspection of potato plants grown to maturity can be performed to check for disease symptoms caused by regulated viruses that are not mechanically transmitted (Table 3).

Since common potato viruses can be present, additional diagnostic indicator hosts may be needed to establish the identity of the virus(es) detected in the initial bioassay. A positive visual diagnosis can be confirmed by other methods appropriate to the suspected pathogen, such as grafting potatoes to appropriate indicator hosts.

Additional non-quarantine potato viruses that rarely occur on potato or that occur in other crops may be of concern in seed potato certification programs. Tests for these viruses may be required, depending on the source of the germplasm. They can be detected by bioassay on appropriate indicator plants (Jeffries 1998).

2.3.1.4 Potato Spindle Tuber Viroid (PSTVd)/Nucleic Acid Based and Bioassay Methods: The reverse-PAGE, dot blot hybridization, and RT-PCR tests can be used to detect PSTVd.

A full or multiple length cRNA probe is required for dot blot hybridization. Positive results with reverse-PAGE and dot blot hybridization may be confirmed by a RT-PCR test. Acceptable primers and probe sources are given

in Table 4. Bioassay on tomato may be used to detect PSTVd or to verify positive laboratory test results.

2.3.2 Acceptable Tests for Regulated Bacteria

Regulated bacteria often are detected by serological tests, such as immunofluorescence microscopy or ELISA, and by PCR. Since serological tests may give false positive results due to cross-reactivity of antibodies, all serologically positive samples should be confirmed. This may be done serologically by testing with a different antibody or by using a different test.

Positive PCR tests should be confirmed by hybridization, RFLP analysis, or sequencing of the amplicon. Other alternatives include isolation followed by characterization of the bacterium. Acceptable antibodies and primers for detecting bacteria are given in Table 4.

2.3.2.1 *Clavibacter michiganensis* subsp. *sepedonicus*

Acceptable tests include ELISA, immunofluorescence microscopy, and PCR. Serological tests must be conducted using antibodies of known specificity for *Clavibacter michiganensis* subsp. *sepedonicus*. Isolated bacteria can be tested by bioassay on eggplant.

2.3.2.2 *Ralstonia solanacearum* race 3

Acceptable tests include ELISA, immunofluorescence microscopy, PCR, and isolation followed by biochemical characterization. Serological tests must be conducted with antibodies of known specificity for *Ralstonia solanacearum* race 3. Two physiological races of *R. solanacearum*, race 1 and 3, are pathogenic on potato, but only race 3 (biovar 2) is a quarantine pathogen. If there is a need for making a biovar determination, biochemical/physiological tests of pure cultures or PCR tests using appropriate primers can be used. Isolated bacteria can be tested by bioassay on tomato.

2.3.2.3 *Erwinia chrysanthemi* potato strains

PCR, ELISA and isolation followed by biochemical characterization can be used to detect *E. chrysanthemi*; antibodies are available for detecting some strains by ELISA. While *E. chrysanthemi* is a pathogen of many plant species and potato strains are poorly characterized the occurrence of this bacterium on potato presents a significant disease threat. All potato germplasm must be free from any strain of *E. chrysanthemi*.

2.3.2.4 *Erwinia carotovora* subsp. *atroseptica*

PCR or isolation followed by biochemical characterization can be used to detect *Erwinia carotovora* subsp. *atroseptica*. ELISA can also be used to detect serogroup I of *E. c. atroseptica*, which predominates in some geographic areas. Antibodies are not available to detect all serogroups of *E. c. atroseptica*. Recent studies suggest that other subspecies of *Erwinia carotovora* can also cause potato blackleg-like disease, all potato germplasm should be free from all strains of pectolytic erwinia.

2.3.3 Acceptable Tests for Regulated Phytoplasmas

The NAPPO Potato Panel has recommended that the NPPOs of the NAPPO member countries eliminate all phytoplasmas from their regulated pest lists. Until such time as the NPPOs act on the Panel's recommendation, testing must be performed and any phytoplasmas detected must be eliminated.

Phytoplasmas can be detected by PCR or visual symptoms on plants. Neither PCR nor visual inspection is adequate to conclusively discriminate among the phytoplasmas. So, test results must be negative for all phytoplasmas.

2.3.4 Test for *In Vitro* Microbial Contaminants

Microbial contamination may mask or indicate the presence of pathogenic or deleterious microorganisms. Therefore, *in vitro* plantlets must be free from all microbial contamination. Contamination can be detected by visual observation of tissue culture medium or by incubating segments of the plantlets aerobically in a liquid growth medium such as Richardson's medium (Table 3) and visually inspecting for turbidity.

3. Minitubers from Approved Pest-Free Places or Sites

3.1 Basic Requirements and Provisions: Minitubers imported for commercial planting must meet the requirements specified in this subsection. They must be certified under the terms of an official seed potato certification program, properly identified by appropriate indicia of certification, and produced in NAPPO member country approved pest-free production site:

- In a protected environment,
- Using clean propagative materials,
- In compliance with a quality assurance system, and
- Where diagnostic and other records are maintained that enable trace-back of potato minitubers through various levels of testing and increase to the original propagative material.

This standard anticipates that officially certified microplantlets, microtubers, or minitubers may move into and within the NAPPO region when requirements are met. The schematic at the beginning of this appendix displays how such movement may occur.

The NPPO may approve pest-free production sites, using the checklist and report forms at the end of this appendix. The NPPO of the importing country is responsible for coordinating all relevant approval activities, including notification of the other two NAPPO NPPOs and performance of an on-site audit to confirm compliance with the requirements. Pest free production site check lists/report forms are provided at the end of this appendix. Representatives of the three NAPPO member countries may collaborate to give approval to pest-free production sites outside of the NAPPO region.

Periodic audits of production sites and operations should be performed to ensure continued compliance with approval conditions. Audit testing may be limited to testing for a single pathogen, provided that pathogen will serve as an adequate surrogate for other pathogens and as a suitable performance check of the production facility's quality assurance system.

3.2 Microplantlet and Microtuber Pest-Free Production Site Requirements

Microplantlets and microtubers must be produced aseptically in tissue culture from pathogen-free parent material as per the requirements for germplasm. In addition, an audit test of microplantlets and microtubers should be performed prior to sale and distribution. The sample size and testing should be adequate to detect any incidence of NAPPO regulated pest infection that exceeds 0.1% at a 95% confidence level.

3.2.1 Parent Material Source and Testing Requirements

Parent material that is to be used for multiplication must originate from a NAPPO NPPO approved pest-free production site. It must be 100% tested and found to be free from NAPPO regulated pests that can be carried in tissue culture (See Table 1 at the end of this appendix). Only a NAPPO member country NPPO or NPPO approved laboratory that meets the criteria outlined in NAPPO RSPM 9, “Accreditation of Laboratories” may perform this required testing. Test results for each lot of parent material microplantlets must be documented.

Operators of an NPPO approved pest-free production site may use their own parent material to produce and sell microplants or microtubers. They also may obtain parent material as officially certified stock from another NPPO approved pest-free production site.

3.2.2 Multiplication Requirements

Multiplication, normally carried out by propagation from nodal cuttings of microplantlets, should be done aseptically. Each microplantlet must be traceable to parental, origin. Microplantlets and microtubers are to be maintained aseptically in growth chambers or rooms. Records shall be maintained to document the number of multiplications and required testing.

3.2.3 Parental Material Monitoring Requirement

Parent material used for commercial production should be sampled and tested annually. A NAPPO member country NPPO approved laboratory that meets the criteria outlined in the NAPPO RSPM 9, “Accreditation of Laboratories” must perform the testing. All testing must be documented.

3.3 Production Requirements for Minitubers

3.3.1 Production Site: Minitubers for importation into or movement within the NAPPO region must be produced in a pest free production site from pathogen-free parent material. Ideally, the production facility should be located in an area that is free from or well isolated from sources of infection by the regulated pests listed in Table 1. However, if the production facility includes adequate physical and operational safeguards against the introduction of regulated pests, geographic isolation is not essential.

3.3.2 Facility Environment: Minitubers must be produced in a pest-free growth room, screenhouse or greenhouse with at least 30-mesh screen and devoid of defects and be constructed to preclude entry of pests and permit decontamination as required. A pest monitoring and control program must be maintained and documented. Facility security must be adequate to prevent unauthorized access.

3.3.3 Growing Media: The kind of growing medium used by the production facility must be

specified and free of all pests. The growing medium must be soil less or heat-treated to eliminate any pests. If heat-treated, the treatment protocol must be specified and pre-approved by a NAPPO member country NPPO. Whether soil less or heat-treated, procedures must be in place to ensure that the growing medium does not become contaminated with weeds and regulated pests (see list in Table 1) in transit, storage and during the growing period.

- 3.3.4 Water and Fertilizer Requirements: Plants for minituber production must be watered from a source that is free from all plant pathogenic microorganisms. Deep-well or treated surface water may be used. If water is treated, the manner of treatment and its efficacy must be specified. Only inorganic fertilizer may be used.
- 3.3.5 Sanitation: The production site must be restricted to minituber production and free from crop residue and weeds. NPPO approved sanitation practices adequate to prevent contamination by plant pests must be employed. Protective foot and outerwear, sanitizing foot baths and hand washing must be required for all personnel.
- 3.3.6 Harvesting and Transport Minitubers harvesting must be in a manner that prevents contamination by regulated pests. Phytosanitary security of the harvested product must be maintained during transportation and storage.
- 3.3.7 Inspection and Testing: Microplantlets grown for minituber production must meet the inspection, testing, disease tolerance and other requirements for nuclear or initial seed as specified by the official, NPPO approved seed potato certification program.
- 3.3.8 Auditing: Upon receipt, the NPPO will take a sample of minitubers for audit testing by the NPPO or a laboratory approved by the NPPO in accordance with NAPPO RSPM 9, "Accreditation of Laboratories". The sample size must be sufficient to ensure the detection of any incidence of regulated pest infection (see list in Table 1) in excess of the official, NPPO approved certification program tolerances at the 95% confidence level.

The audit test may be limited to testing for a single pathogen, provided that testing for that pathogen will serve as an adequate surrogate for other pathogens and as a suitable check of the efficacy of the production facility's quality assurance program and compliance with the official, NPPO approved seed potato certification program. Regulatory action in response to a positive test result for a surrogate pathogen is the responsibility of the NPPO.

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Table 1 Quarantine and Regulated Non-Quarantine pests that may infect *in vitro* potato plantlets.

Viroid

Potato spindle tuber viroid

Viruses

Andean potato latent virus

Andean potato mottle virus

Arracacha virus B – oca strain

Beet curly top virus

Pea Streak Virus, Alfalfa Enation Strain

Potato Black Ringspot Nepovirus

Potato deforming mosaic virus

Potato latent virus

Potato leaf roll virus

Potato mop-top virus

Potato virus A

Potato virus M

Potato virus S

Potato virus T

Potato virus U

Potato virus V

Potato virus X

Potato virus Y^O, Y^C, Y^N, and Y^{NTN}

Potato yellow vein virus

Potato yellowing virus

Tobacco Necrosis Virus

Tobacco rattle virus

Tobacco ringspot virus – calico strain

Tomato black ring virus

Bacteria

Clavibacter michiganensis subsp. *sepedonicus*

Erwinia carotovora subsp. *atroseptica*

Erwinia chrysanthemi (potato strain)

Ralstonia solanacearum race 3, biovar 2

Phytoplasma

Table 2. Mechanically transmitted viral quarantine pests and indicator bioassay hosts (Jeffries, C.J. 1998. FAO/IPGR Technical guidelines for the Safe Movement of Germplasm. No. 19. Potato.)

Virus	Bioassay host
Andean potato latent virus, <i>Tymovirus</i>	<i>Nicotiana bigelovii</i> ; <i>Nicotiana benthamiana</i>
Andean potato mottle virus, <i>Comovirus</i>	<i>Nicotiana bigelovii</i> ; <i>Nicotiana clevelandii</i>
Arracacha B virus, Oka strain, <i>Nepovirus</i>	<i>Chenopodium amaranticolor</i> ; <i>Chenopodium murale</i>
Potato virus T, <i>Trichovirus</i>	<i>Chenopodium amaranticolor</i> ; <i>Chenopodium quinoa</i>
Potato virus U, <i>Nepovirus</i>	<i>Chenopodium quinoa</i> ; <i>Nicotiana tabacum</i> cv. White Burley
Potato virus V, <i>Potyvirus</i>	<i>Nicotiana debneyi</i> ; <i>Lycopersicon esculentum</i>
Potato virus Y ^C <i>Potyvirus</i>	<i>Nicotiana tabacum</i> cv. White Burley; <i>Nicotiana benthamiana</i>
Potato yellowing virus, <i>Alfamovirus</i>	<i>Capsicum annuum</i> , <i>Nicotiana tabacum</i> cv. Samsun
Tobacco ringspot virus, calico strain (=Potato Black Ring Spot), <i>Nepovirus</i>	<i>Chenopodium amaranticolor</i> ; <i>Nicotiana benthamiana</i>
Tomato black ring virus, <i>Nepovirus</i>	<i>Chenopodium amaranticolor</i> ; <i>Nicotiana tabacum</i> cv. White Burley

Table 3. Non-mechanically transmitted viral quarantine pests that infect potato. These viruses are detected by observing mature plants for symptoms.

Virus	Symptom
Beet curly top virus, <i>Curtovirus</i>	Terminal leaflets cupped, misshapen and chlorotic.
Potato deforming mosaic virus, <i>Begomovirus</i>	Leaf deformation and yellow mosaic.
Potato yellow vein virus, <i>Crinivirus</i>	Veins initially bright yellow, leaves gradually becoming yellow.

Table 4: Acceptable antibodies, primers and probes for serological and molecular detection of potato quarantine and regulated prokaryote and viroid pests.

Pest	Test	Detection Reagent	Reference
<i>C. m. sepedonicus</i>	Immuno-Fluorescence	Monoclonal 9A1 (Agdia)	De Boer & Wieczorek (1984)
	ELISA	Monoclonal 1H3 (Agdia)	De Boer et al (1988)
	PCR	Primers: Sp1f, Sp5r	Li & De Boer (1995)
		Primers: CMS6, CMS7	Schneider et al (1993)
		Primer sets: Cms50 Cms72, Cms85	Mills et al (1997)
	Nested PCR	Primers: CMSIF1, CMSIR1 CMSIF2, CMSIR2	Lee et al (1997)
	RT-PCR	Primers/probe: Cms 50-2F Cms133R, CMS 50-53T	Schaad et al (1999)
<i>E. chrysanthemi</i>	ELISA	Monoclonal 6A6	Singh et al (2000)
	PCR	Primers: ADE1, ADE2	Nassar et al (1996)
<i>E. c. atroseptica</i>	ELISA	Monoclonal 4F6 (Agdia)	De Boer and McNaughton
	PCR	Monoclonal 6G6 (IVIA)	Gorris et al (1994)
		Primers: ECA1f, ECA2r	De Boer and Ward (1995)
		Primers: Y45, Y46 (Probelia)	Frechon et al (1995)
<i>R. solanacearum</i>	Immuno- Fluorescence	Polyclonal: IACR-276; Adgen 1091; IPO-9523	Elphinstone et al (2000)
	ELISA	Monoclonal 6.9-D5	Singh & Trevors (2001)
		Polyclonal IACR-278; Adgen 1091	Elphinstone et al (2000)
	PCR	Primers: PS96-H, PS96-I	Seal et al (1992)
		Primers: OLI-1, Y-2	Seal et al (1993)
	RT- PCR	Primers/probe: B2-I-F, B2-II-R, B2-P	Weller et al (2000)
	Isolation	Medium: SMSA	Elphinstone et al (2000)
PSTVd	Probe	Agdia	
	RT-PCR	Primers: unnamed	Shamloul et al (1997)
Phytoplasma	PCR	Primers: unnamed	Ahrens & Seemuller (1992)
	Nested PCR	Primers: R16MF2 R16MR1, R16F2N, R16R2	Gundersen & Lee (1996)
		Primers: P1, TINT R16F2, R16R2	Martinez-Soriano et al (1999)
Microbial Contaminants	Isolation	Richardson's medium	Richardson (1957)

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NAPPO Pest-Free Production Site Checklists and Report Forms

Appendix 4 provides for representatives of the three NAPPO member country national plant protection organizations (NPPO) to collaborate in the approval of pest-free production sites, using checklist and report forms. In order to ensure transparency and uniformity, the following three-part checklist/report form is provided. Part A is for use in the approval of aseptic culture production sites where microplantlets and microtubers are produced. Part B is for the approval of the environmental or security controls at either an aseptic culture or microtuber production site. Part C is for use in the auditing or monitoring of crops being grown in microtuber production sites.

Part A. Checklist/Report Form for Facilities Producing Plants in Tissue Culture

Facility Owner/Operator Title, Name		Telephone number		
Facility Owner/Operator Address		Facsimile Number / E-mail Address		
Facility Owner/Operator Number		Date of Application (month/day/year)		
Name and Title of Inspector(s)		Telephone number(s)/E-mail Address(es) of inspector(s)		
Criteria		Yes	No	Comments
Application lists all varieties being multiplied, and documentary proof of origin provided				
Producer maintains adequate information system and records for: varietal identification, initiation/propagation dates, origin, sample submission dates, test results, and certification numbers (where applicable)	Variety ID			
	Initiation date			
	Origin			
	Testing dates			
	Cert ⁿ number			
All material produced from tissue culture sources tested and found to be free from quarantine and regulated non-quarantine pests that may infect in vitro potato plantlets (Table 1 of Appendix 4): BRR, PVA, PVM, PVS, PVX, PVY, PLRV, PSTVd, and PotLV within the last 12 months.				
Procedures manual exists, is implemented, and recognized aseptic procedures used				
Aseptic environment maintained in required areas				
Laminar flow hood functioning (insert date of last test certificate)				
Material and containers visibly free from bacteria, viruses, saprophytic contamination or other diseases				
All containers capped				
All containers labeled with variety and traceable identification number				
Physical separation exists between material being initiated, banked, and/or multiplied				
Banked stock clearly labeled as such				
Facility meets requirements?				<u>Overall or additional comments</u>
Facility Operator's signature: _____				
Inspector's signature: _____				
Date of inspection /signature: _____				

Part B. Pest Free Production Site (Greenhouse) Pre-Planting Environment Checklist/Report Form

Facility Owner/Operator Title, Name		Telephone number	
Facility Owner/Operator Address		Facsimile Number / E-mail Address	
Facility Owner/Operator identifier		Date of Application (month/day/year)	
Name and Title of Inspector(s)		Telephone number(s)/E-mail Address(es) of inspector(s)	
Name/Telephone Number of Alternate Contact Person:			
Number of protected environment units producing potato microplantlets/mini tubers:			
This inspection report relates to production unit number:			
Criteria	Yes	No	Comments
Facility is aphid-proof at all possible entry points			
Facility (and all containers) disinfected and free of solanaceous plants or debris			
Entrance sanitizing foot-bath present			
Adequate double door entrance			
Soil-free or treated medium will be used			
Separation between medium and untreated soil			
Insect monitoring program, sanitation practices and information system established			
Facility meets requirements?			<u>Overall or additional comments</u>
Facility Operator's signature: _____			
Inspector's signature: _____			
Date of inspection /signature: _____			

Part C. Pest Free Production Site Growing Crop Checklist/Report Form

Facility Owner/Operator Title, Name		Telephone number	
Facility Owner/Operator Address		Facsimile Number / E-mail Address	
Facility Owner/Operator Number		Date of Application (month/day/year)	
		/	
Name and Title of Inspector(s)		Telephone number(s)/E-mail Address(es) of inspector(s)	
Alternate Production Site Contact Person:		Crop:	' 1st ' 2nd ' 3rd
Number of protected environment units producing potato microplantlets/minitubers:			
This inspection report relates to production unit number:			
Criteria	Yes	No	Comments
Application lists all varieties present, and documentary proof of origin provided			
All lots produced from material tested and found to be free from all quarantine and regulated non-quarantine pests that may infect in vitro potato plantlets (Table 1 of Appendix 5): bacterial ring rot, PVA, PVM, PVS, PVX, PVY, PLRV, PSTVd, and PotLV within the last 12 months.			
Record keeping and traceback system in place			
Effective sanitation practices plus disease and insect monitoring program utilized			
Facility is constructed to preclude entry of pests and permit decontamination as required			
Sanitation protocol including hand wash and sanitized foot ware and outerwear in place			
Facility security prevents unauthorized access			
All medium used is soil-free or heat treated			
Water is free of pathogenic microorganisms			
Inorganic fertilizer used			
Varieties/clones separated by physical barriers			
Visibly free from varietal mixtures			
Free from non-nuclear potatoes, other solanaceous plants, or weeds			
Free from observable symptoms of disease			
Free from observable presence of, or signs of, aphids			
Harvesting method prevents contamination by regulated pests			
Growing crop meets program requirements			Overall or additional comments
Facility Operator's signature: _____			
Inspector's signature: _____			
Date of inspection /signature: _____			

Appendix 5

Pre-Shipment Testing for PVY^N

1. Introduction

Systems approaches can be employed to manage risk to an acceptable level. To this end, the United Nations Food and Agricultural Organization's International Standard for Phytosanitary Measures (ISPM) 14, "The use of integrated measures in a systems approach for pest risk management" describes "systems approaches" for pest risk management. This appendix primarily deals with the testing component of a PVY^N risk management system. The fundamental framework for PVY^N risk management among NAPPO member countries includes:

Official certification of the candidate seed lot under the terms of a limited generation seed potato certification scheme. Seed potato certification is the basic or foundation component of the system. PVY^N monitoring during the growing season.

Pre-shipment testing of eligible seed lots presented by growers for phytosanitary certification by officials of the exporting country's national plant protection organization.

Other alternatives for reducing PVY^N risk to an acceptable level include 1) trade in basic stock including plantlets, minitubers, etc. produced by the exporting country approved laboratory or greenhouse facilities, as described in the requirements in Appendix 4 of this standard and 2) the shipment of seed potatoes from PVY^N pest free areas approved pursuant to the NAPPO RSPM 1, "Pest Free Areas".

2. Background Information

Potato Virus Y pathovar N (PVY^N) and the PVY^N tuber necrosis pathovar (PVY^{NTN}), together with PVY^{NTN}-like viruses are quarantine pests for the NAPPO region. The harm that they can cause to tobacco and certain other solanaceous crops varies with the crop, cultivar, and disease incidence and severity. These pathogens can be spread mechanically, by aphids during the growing season, and moved over long distances by the transport and planting of infected live host plant material including potato.

Certain PVY^{NTN}-like viruses do cause symptoms on potato, including tuber necrosis in sensitive cultivars. PVY^N and PVY^{NTN}-like isolates are closely related but may be differentiated from PVY^O.

There is evidence that PVY^N and PVY^O isolates can recombine to form PVY^{NTN}-like isolates. As a result, some PVY^N isolates are genetically similar to PVY^{NTN}, but their pathogenicity on potato is not well known or described. Nevertheless, these isolates can be detected in the laboratory using currently available diagnostic protocols.

While pre-harvest testing of potato foliage is a useful monitoring or survey procedure, it is not an appropriate method for determining the absence of PVY^N and PVY^{NTN}-like virus isolates in seed potato shipments because:

- 2.1 Distribution of virus in the plant is uneven,
- 2.2 Virus titre in aging plants is low, and
- 2.3 Late season infections cannot be detected.

Therefore, a sample of tubers collected from the seed potato crop must be tested.

3. PVY^N Pre-Shipment Testing

3.1. Sample Collection and Sample Size

- 3.1.1 The probability of detecting PVY^N and PVY^{NTN}-like virus in a seed lot is limited by sample size, virus incidence, and diagnostic methodology.
- 3.1.2 Only persons officially approved by exporting country's national or provincial/state plant protection organization may collect samples.
- 3.1.3 Samples must be identified in a manner that enables trace-back to the specific seed lot from which they were collected.
- 3.1.4 Samples must be protected during collection, transport, and storage from harm that might interfere with the detection of PVY^N.
- 3.1.5 Post-harvest sampling must be a minimum of 400 tubers randomly collected from the seed lot at harvest or from storage. This sample size provides a 98% confidence level for detection of a virus incidence of 1%.

3.2 Laboratory Diagnostic Methodologies

Either the Enzyme Linked Immunosorbent Assay (ELISA) or Reverse Transcription-Polymerase Chain Reaction (RT-PCR) may be used for testing. The prerequisites, regardless of which test methodology is used, are:

- 3.2.1 Tests must be conducted according to a standard protocol agreed upon by the importing and exporting country's national plant protection organizations (NPPOs).
- 3.2.2 Laboratory tests must be done under the auspices of a qualified plant pathologist or within a quality assurance system approved by the importing and exporting country NPPOs.
- 3.2.3 Positive and negative control samples must be run in conjunction with test samples.

The type of tissue that can be used for testing for these test procedures and number of samples that may be bulked into a single composite sample are shown in Table 1.

Table 1. Approved testing strategies for PVY^N and PVY^{NTN}-like viruses.

Test	Dormant tubers	Tuber Sprouts	Plant Leaves
ELISA	Not permitted	Individually	Composites of 25
RT-PCR	Composites of 50	Composites of 100	Composites of 200

4. ELISA Testing

ELISA is a valid methodology for testing tuber sprouts and leaves from plants grown from sample tubers. It is not valid for the testing of dormant tubers.

There are a number of acceptable ELISA protocols. However, a positive/negative threshold must be identified in the ELISA protocol being used.

The following two monoclonal antibodies are highly specific for PVY^N and PVY^{NTN} strains: 1F5 and the Scottish 'Rose' antibodies. These antibodies are available commercially (e.g. Agdia, Adgen, etc).

Despite the high specificity of the foregoing antibodies, it is recognized that they may react with some isolates of PVY^O.

Also, mutations and genetic recombination between PVY strains may occur and not react with these antibodies. Hence a positive ELISA result must be confirmed by RT-PCR using one of the primer sets identified in this appendix to unequivocally identify an isolate as being of the PVY^N and PVY^{NTN} type.

While a positive bioassay result on tobacco is a valid confirmatory test for PVY^N, bioassays are infeasible because none are completely reliable for *all* PVY^{NTN}-like viruses, the cost is high, and they require a long time to perform them. Hence a positive ELISA result must be confirmed by RT-PCR using one of the primer sets identified in this appendix to unequivocally identify an isolate as being of the PVY^N and PVY^{NTN} type.

5. RT-PCR Testing

Although the RT-PCR is more sensitive than ELISA, sensitivity is not normally a limiting factor for detecting PVY^N and PVY^{NTN}-like viruses. Two different sets of primers may be used for the RT-PCR. They are primer sets A/S4 (primer A to be used for RT) and primer set PY13/PY14 (primer PY13 being used for RT) as per Table 2 (Nie and Singh 2002, Xu 2002, Xu and Nie 2002).

RT-PCR methodology is valid for dormant tubers, tuber sprouts, and leaves from plants grown from sample tubers.

Certain cultivars require that a specific RNA extraction protocol be used (Singh et al 2002). The temperature profile of the PCR assay must conform to a published protocol or be confirmed by supporting data. Due to varying thermal characteristics among thermocyclers, the PCR protocol must be confirmed for the thermocycler used for test samples.

A positive RT-PCR result must be confirmed by a repeat RT-PCR test on fresh tissue from the same sample.

Table 2. Primer sequences

Primer Set	Primer base sequence (5'-3')	PCR product size
A	CATTTGTGCCCAATTGCC	443 bp
S4	GGTGAAGCTAATCATGTCAAC	
PY13	TTCTAATCTGTGCTCTGGCAG	315 bp
PY14	CATTACGCTCTCATTAAGGAGT	

6. Sample Preparation

6.1. Dormant Tubers

Dormant tuber tissue may be tested only by RT-PCR.

Tissue must be taken from both the bud end and the stem end from each tuber.

Tissue from up to 50 tubers may be bulked and tested as a composite sample.

6.2. Tuber Sprouts

Any method of breaking tuber dormancy is acceptable.

Sprouts must be taken from the bud end of the tuber.

Two sprouts must be tested from each tuber but they may be combined.

Sprouts must be at least 0.5 cm but not more than 6.0 cm long.

Sprouts from different tubers may not be bulked as a composite sample for ELISA.

Sprouts (100) from up to 50 tubers may be bulked as a composite sample for RT-PCR.

6.3. Leaves

Plants must not be etiolated and at least 15 cm tall.

Leaves from plants grown from sample tubers may be tested.

The testing of a single, fully expanded leaf from the mid-region of the stem of each plant is acceptable.

Tissue from up to 25 leaves may be bulked as a composite sample for ELISA.

Tissue from up to 200 leaves may be bulked as a composite sample for RT-PCR.

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