

23 August 2016

**Economic Commission for Europe**

Steering Committee on Trade Capacity and Standards

**Working Party on Agricultural Quality Standards**

**Specialized Section on Standardization  
of Seed Potatoes**

**Forty-third session**

Geneva, 31 August (pm) – 2 September 2016

Item 5 of the provisional agenda

**PCR techniques for virus testing**

**PCR techniques for virus testing**

The following revised version of the survey was prepared by the Rapporteur (Finland), with input from Australia, the Czech Republic, and Sweden. The Specialized Section is invited to review and comment.

## Survey of potato virus testing methods that are associated with seed certification

### Introduction

The goal of the UNCE Seed Potato Certification Standard is to act as a world reference intended to facilitate fair international trade by:

- Creating a harmonized commercial quality certification system
- Promoting its use
- Defining harmonized quality requirements for seed potatoes.
- 

To reach this goal the UNCECE Standard covers the following requirements controlled by certification:

- Varietal identity and purity
- Genealogy and traceability
- Diseases and pests affecting commercial quality or yield
- External quality and physiology
- Sizing and labelling.

In maintaining the Standard, it is vital that the current practices used in seed potato certification are reviewed and the Standard updated.

### Purpose of this survey

The purpose of this survey is to

1. Capture information from around the world regarding potato virus testing methods that are used to support decisions in seed potato certification.
2. Develop a comparative list of the virus testing methods which can be used as a reference/guide for all seed potato certification authorities.
3. To determine how the UNECE standard should reflect the current practices of virus testing that is associated with seed potato certification

The data generated will be made publicly available through the UNECE seed program website

### General Information

Country

Name of seed potato certification authority (Designated Authority)

Date of completion

**Laboratory virus testing that is associated with seed potato certification**

1. Please state the reason for virus testing potato crops in your country and who is responsible for the testing:

Virus testing is conducted by:	Reason for virus testing			
	Compulsory for all seed crops as part of certification	Compulsory for all seed crops with exemptions under certain conditions	Voluntary	Not conducted
Your organisation				
Government Laboratory				
University/Research Institute				
Private Laboratory				
Laboratory in other country				
Other				

2. Please explain below if virus testing is exempt under certain conditions (i.e. aphid status, haulm killing time, varieties etc.)

3. The criteria to choose the laboratory (tick all that apply)

i.	<input type="checkbox"/>	The efficacy and reliability of virus tests
ii.	<input type="checkbox"/>	The rapidity of virus tests
iii.	<input type="checkbox"/>	The price of the virus tests
iv.	<input type="checkbox"/>	Third party accreditation
v.	<input type="checkbox"/>	Other criteria <input type="text"/>
vi.	<input type="checkbox"/>	No possibility to choose the laboratory

4. Type of potato virus testing

i.	<input type="checkbox"/>	Potato leaf testing during growing season
ii.	<input type="checkbox"/>	Post -harvest virus testing (tuber samples)
iii.	<input type="checkbox"/>	Both
iv.	<input type="checkbox"/>	Other <input type="text"/>

5. Seed categories tested, virus tested and the method for leaf samples

Virus	Sample	PBTC	PBTC	PB	PB	Basic	Basic	Certified	Certified
			method		method		method		method
PLRV	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVY	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVA	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVX	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVS	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA

Comment [KH1]: visual assessment?

Comment [nc2]: Not sure what this references?

Potato virus survey associated with seed potato certification

									<input type="radio"/> Other
PVM	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
Other	field/leaves	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other

If other methods are also used, please specify.

6. Seed categories tested, virus tested and the method for tuber samples

Comment [KH3]: visual assessment?

Virus	Sample	PBTC	PBTC	PB	PB	Basic	Basic	Certified	Certified
			method		method		method		method
PLRV	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVY	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVA	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVX	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVS	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
PVM	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other
Other	tuber	Yes No NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> NA	<input type="radio"/> PCR <input type="radio"/> ELISA <input type="radio"/> Bioassay <input type="radio"/> Other

If other methods are also used, please specify.

## Sampling

7. Please specify the sample size for each set of circumstances described above.  
Leaf samples

**Comment [KH4]:** collected sample size or tested sample size or both?

Virus	PBTC	PB	Basic	Certified
PLRV				
PVY				
PVA				
PVX				
PVS				
PVM				
Other				

8. Please specify the sample size for each set of circumstances described above.  
Tuber samples

Virus	PBTC	PB	Basic	Certified
PLRV				
PVY				
PVA				
PVX				
PVS				
PVM				
Other				

## Timing of sampling

If leaves are sampled during the season:

9. When are leaves sampled in relation to the age of the crop?

10. How soon are the leaf samples processed for virus testing after sampling?

For Post-harvest tuber testing:

11. When are tubers sampled in relation to harvest?

12. How soon are tuber samples processed for virus testing after sampling?

OR instead questions 9-12 only one question (13) of timing of sampling

13. Timing of tuber sampling in relation to crop harvest

i.		Please enter number before units	
ii.	<input type="checkbox"/>	<input type="text"/>	Days
iii.	<input type="checkbox"/>	<input type="text"/>	Weeks
iv.	<input type="checkbox"/>	<input type="text"/>	Months

### Test methods

14. Please specify the official test methods used by the laboratory

Test method	Sample				
	Leaves (growing crop)	Direct tuber	Leaves grown from tubers (glasshouse)	Sprouts grown from tubers	Other
PCR					
ELISA					
Visual assessment					
Bioassay					
Other					

OR instead of question 14 separate questions 15-18



15. Official potato virus testing method used by laboratory

v.	<input type="checkbox"/>	PCR
vi.	<input type="checkbox"/>	ELISA
vii.	<input type="checkbox"/>	Visual Assessments
viii.	<input type="checkbox"/>	Bioassay
ix.	<input type="checkbox"/>	Other <input type="text"/>

16. Other potato virus testing methods used by laboratory

i.	<input type="checkbox"/>	PCR
ii.	<input type="checkbox"/>	ELISA
iii.	<input type="checkbox"/>	Other <input type="text"/>

17. Samples used for PCR testing

i.	<input type="checkbox"/>	Tubers
ii.	<input type="checkbox"/>	Leaves from field
iii.	<input type="checkbox"/>	Leaves grown from tubers (greenhouse)
iv.	<input type="checkbox"/>	Bioassay
v.	<input type="checkbox"/>	Other <input type="text"/>

18. If tuber testing is conducted, at what stage is it usually tested?

i.	<input type="checkbox"/>	Direct tuber: Number of weeks after harvest <input type="text"/>
ii.	<input type="checkbox"/>	Chitted tuber (eyes open, no green tissue)
iii.	<input type="checkbox"/>	Green sprouts
iv.	<input type="checkbox"/>	Other <input type="text"/>

19. If ELISA is used in the laboratory, how was it developed

i.	<input type="checkbox"/>	In-house developed method
ii.	<input type="checkbox"/>	Commercial kit method (please specify supplier) <input type="text"/>
iii.	<input type="checkbox"/>	Other <input type="text"/>

20. How are the samples pooled for ELISA testing? ☐ Yes ☐ No

a. If Yes, what is the total number of subsamples?

21. If PCR is used in the laboratory, how was it developed

i.	<input type="checkbox"/>	In-house developed method
ii.	<input type="checkbox"/>	Commercial kit method (please specify supplier) <input type="text"/>
iii.	<input type="checkbox"/>	Other <input type="text"/>

22. Are the PCR primer sequences publicly available for use? ☐ Yes ☐ No

Please provide reference for the primer sequences

23. How are the tubers/leaves pooled/bulked for PCR testing? ☐ Yes ☐ No

a. If Yes, what is the total number of subsamples for

Tuber samples:

Leaf samples:

b. How are the results statistically interpreted for use in certification e.g. ISTA seedcalc?

## Results

24. How does the DA use the lab result to determine the classification of the crop:

- I. Please supply the classification table, including the how the results determine the class of the crop
- II. Other, if the result of the testing does not directly affect the class of the seed lot. Please specify how the virus testing information is used.

## Quality Assurance

25. Is the laboratory accredited/approved for the above tests?

☐ Yes ☐ No

a. If yes by which accreditation/approval body?

- b. If not, do the laboratory have an internal Quality Control system? ☐ Yes ☐ No  
c. Has the laboratory validated their PCR virus testing method?  
☐ Yes ☐ No ☐ In progress

26. Have the PCR methods used for certification been independently validated/accredited?

- a. For Tuber testing: ☐ Yes ☐ No  
b. For Leaf testing: ☐ Yes ☐ No

27. Does the laboratory participate in any ring tests/ proficiency tests of potato virus testing?

- ☐ Yes ☐ No  
a. If yes, with whom?

28. Does the Designated Authority audit the laboratory and testing procedures?

- a. Laboratory: ☐ Yes ☐ No  
b. Testing Procedures: ☐ Yes ☐ No

29. How often is the laboratory and its testing procedures audited?

30. In your experience, do the different testing methods give comparable results?

- I. ELISA and PCR: ☐ Yes ☐ No ☐ Don't Know ☐ Not applicable  
II. ELISA and visual inspection of the progeny:  
☐ Yes ☐ No ☐ Don't Know ☐ Not applicable  
III. PCR and visual inspection of the progeny:  
☐ Yes ☐ No ☐ Don't Know ☐ Not applicable  
IV. Other

Please explain your answer:

31. Have your clients provided feedback on the virus test methods or results?

- ☐ Yes ☐ No Provide details

32. Other comments?

- Include example table from NL (virus result interpretation)