ECE/CTCS/WP.7/GE.6/2020/INF.2
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Economic Commission for Europe

Steering Committee on Trade Capacity and Standards

Working Party on Agricultural Quality Standards

Specialized Section on Standardization of Seed Potatoes

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Item 5 of the provisional agenda
Draft survey on bacterial testing methodologies

Update to revised Survey of testing methods for bacterial pathogens of potato that are associated with seed certification

The following document, received from the delegation of the United States, is an update to the bacterial pathogen survey (ECE/CTCS/WP.7/GE.6/2020/5).

Introduction

The goal of the UNECE 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 UNECE 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 (http://www.unece.org/trade/agr/standard/potatoes/pot_e.html).

Purpose of this survey

The purpose of this survey is to

1. Capture information from around the world regarding potato bacterial pathogen testing methods that are used to support decisions in seed potato certification.
2. Develop a comparative list of the bacteria 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 testing for bacterial pathogens that are associated with seed potato certification

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

Target Audience

This survey is intended to be completed by the authority responsible for seed certification. The authority may wish to liaise with testing services in order to complete the survey.

General Information

Country

Name of seed potato certification authority

Bacterial Pathogens Survey:

Questions on Pathogens Causing Blackleg Disease of Potato

1. Is Blackleg a disease problem in potato for certification in your country?

☐	Yes
☐	No

2. Do you have *Pectobacterium* sp. associated with potato blackleg in your country?

☐	Yes
☐	No

If yes, specify which Pectobacteria are known to occur:

☐	*Pectobacterium atrosepticum*
☐	*Pectobacterium carotovorum* subsp. *carotovorum*
☐	*Pectobacterium carotovorum* subsp. *brasiliense*
☐	*Pectobacterium carotovorum* subsp. *odorifera*
☐	*Pectobacterium wasabiae*
☐	*Pectobacterium parmentieri*
☐	Other
☐	Do not know species

3. Do you have *Dickeya* spp. associated with potato blackleg in your country?

☐	Yes
☐	No

If yes, what *Dickeya* species are known to occur?

☐	*Dickeya dianthicola*
☐	*Dickeya solani*
☐	*Dickeya zea*
☐	Other
☐	Do not know species

4. Potato blackleg laboratory testing in your country is: (Check all that apply)

Pectobacterium spp.	*Dickey*a spp.	
☐	☐	Compulsory for all crops as part of seed potato certification
☐	☐	Compulsory for all crops with exemptions* under certain conditions
☐	☐	Voluntary by grower
☐	☐	Compulsory if found in field
☐	☐	For confirmation of visual symptoms
☐	☐	Not done

*Exemptions may include seed class, generation, variety

5. Blackleg testing is conducted according to the following criteria (Check all that apply)

Pectobacterium spp.	*Dickeya* spp.	
☐	☐	Origin of seed
☐	☐	Variety
☐	☐	By Class
☐	☐	Crop Rotation
☐	☐	Irrigation source
☐	☐	Customer request
☐	☐	Inspection history/findings
☐	☐	Surveillance
☐	☐	Symptomatic plants

6. Blackleg testing is done by:

☐	Your organization
☐	Other governmental laboratory
☐	University or research institute
☐	Private laboratory
☐	Laboratory in other country
☐	Laboratory approved by the CA

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

☐	The reliability of tests
☐	The rapidity of tests
☐	The price of the tests
☐	Third party accreditation
☐	No possibility to choose
☐	Mandatory requirement to use a particular lab

8.1 Type of potato tissue tested for blackleg pathogens (check all that apply)

☐	Microplants
☐	Stems during growing season
☐	Tubers

8.2 Is enrichment used prior to conducting specific tests:

☐	Yes (see 8.4)
☐	No (see 8.5)

8.3. Is incubation of tubers at controlled temperature and humidity used to enhance populations prior to conducting specific tests on tubers?

☐	Yes (see 8.4)
☐	No (see 8.5)

8.4. What methods are used for testing for pathogens causing blackleg disease (with enrichment or incubation)

Pathogen	Type of potato tissue tested		
	Microplants	Stems	Tubers
	Method		
Pectobacterium spp.	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other
Dickeya spp.	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other

8.5. What methods are used for testing for pathogens causing blackleg disease (without enrichment or incubation)

Pathogen	Type of potato tissue tested		
	Microplants	Stems	Tubers
	Method		
Pectobacterium spp.	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other
Dickeya spp.	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other

8.6. Please specify the sample size required for microplants, stems and tubers for each blackleg pathogen.

	microplants	Stems	Tubers
Pectobacterium spp.	☐1-50 ☐51-100 ☐101-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600
Dickeya spp.	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600*	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600

*reference

8.7. What is the subsample size for analysis of the samples in question 8.6

	Subsample size
Pectobacterium spp.	☐5 ☐10 ☐20 ☐25 ☐50 ☐>50
Dickeya sppi	☐5 ☐10 ☐20 ☐25 ☐50 ☐>50

9. If tuber testing is conducted, what part of the tuber is sampled (tick all that apply)

☐	Peel taken at heel/stolon end
☐	Plug taken at heel/stolon end
☐	Peel taken at Rose end
☐	Plug taken at rose end

10.1 If ELISA is used in the laboratory, how was it developed?

☐	In-house
☐	Commercial Kit
☐	Not Used

10.2 If an in-house ELISA method is used, are you willing to share the method?

☐	Yes*
☐	No

*To receive information contact

10.3 Do you use a commercial ELISA kit method.

☐	Yes
☐	No

If yes, please specify supplier.

10.4 Other ELISA Method (If yes, fill in blank)

11.1 If PCR is used in the laboratory, what is the nucleic acid extraction protocol?

☐	In-house
☐	Commercial Kit
☐	Not Used

11.2 If nucleic acid extraction protocol was developed in-house, what is the reference and/or contact information?

11.3 If an in-house nucleic acid extraction method is used, are you willing to share the protocol

☐	Yes
☐	No

Please supply contact for protocol

11.4 If a commercial kit method is used for nucleic acid extraction, please list the kit name and supplier.

11.5 Other nucleic acid extraction Method (If yes, fill in blank with description)

11.6 Are the stems/ tubers pooled/bulked for PCR testing?

☐	Yes
☐	No

11.7 Please specify the sample size required for stems and tubers.

	Stems	Tubers
Pectobacterium spp.	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600
Dickeya spp.	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600*	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600

11.8 What is the subsample size for analysis of the samples in question 11.7?

	Subsample size
Pectobacterium spp.	☐5 ☐10 ☐20 ☐25 ☐50 ☐>50
Dickeya sppi	☐5 ☐10 ☐20 ☐25 ☐50 ☐>50

11.9 Are the PCR primer sequences published?

☐	Yes
☐	No

Please provide references for the primer sequences

11.10 If primer sequences are not published, would laboratory be willing to share sequences and protocols

☐	Yes
☐	No

Contact for primer sequences and protocol

12 Is sequencing used to determine speciation?

Pectobacterium spp.	*Dickeya* spp.	
☐	☐	Yes
☐	☐	No

13 If selective media is used to isolate blackleg pathogens:

Please provide selective media information for *Pectobacterium* spp.

Please provide selective media information for *Dickeya* spp.

Questions on *Clavibacter michiganensis subsp. Sepedonicus,* the Causal Agent of Bacterial Ring Rot

14 Is *Clavibacter michiganensis subsp. sepedonicus* (CMS) known to occur in your country:

☐	Yes
☐	No

15 CMS testing in your country is:

☐	Compulsory for all crops as part of seed potato certification
☐	Compulsory for all crops with exemptions under certain conditions*
☐	Voluntary by grower
☐	For confirmation of visual symptoms
☐	Surveillance (do above for blackleg)
☐	Not done

*Exemptions may include seed class, generation, variety

16 CMS testing is conducted according to the following criteria (Check all that apply)

☐	Origin of seed
☐	Variety
☐	Class
☐	Crop Rotation
☐	Irrigation source
☐	Customer request
☐	Inspection history/findings
☐	Surveillance
☐	Symptomatic plants

17 CMS testing is done by:

☐	Your organization
☐	Other governmental laboratory
☐	University or research institute
☐	Private laboratory
☐	Laboratory in other country
☐	Laboratory approved by the CA

18 The criteria to choose the laboratory for CMS (tick all that apply)

☐	The reliability of tests
☐	The rapidity of tests
☐	The price of the tests
☐	Third party accreditation
☐	No possibility to choose
☐	Mandatory requirement to use a particular lab

19 Type of potato tissue tested for CMS (check all that apply)

☐	Microplants
☐	Stems during growing season
☐	Tubers

21.1 Is enrichement used to enhance populations of CMS prior to conducting specific tests on tubers?

☐	Yes (see 21.3)
☐	No (see 21.4)

21.2 Is incubation of tubers at controlled temperature and humidity used to enhance populations of CMS prior to conducting specific tests on tubers?

☐	Yes (see 21.3)
☐	No (see 21.4)

21.3 What methods are used for testing for CMS (with enrichment or incubation)?

	Type of potato tissue tested		
	Microplants	Stems	Tubers
Pathogen	Method		
Clavibacter michiganensis subsp. sepedonicus	○ Not Tested ○ PCR ○ ELISA ○ IF ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ IF ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ IF ○ Selective Media ○ Other

21.4 What methods are used for testing for CMS (without enrichment or incubation)?

Pathogen	Type of potato tissue tested		
	Microplants	Stems	Tubers
	Method		
Clavibacter michiganensis subsp. sepedonicus	○ Not Tested ○ PCR ○ ELISA ○ IF ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ IF ○ Selective Media ○ Other	○ Not Tested ○ PCR ○ ELISA ○ IF ○ Selective Media ○ Other

22.1 Please specify the sample size required for microplants, stems and tubers for CMS

	microplants	Stems	Tubers
Clavibacter michiganensis subsp. sepedonicus	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600

22.2 What is the subsample size for analysis of the samples in question 22.1

Subsample size
☐50 ☐100 ☐200 ☐400

23 If tuber testing is conducted for CMS, what part of the tuber is sampled (tick all that apply)

☐	Peel taken at heel/stolon end
☐	Plug taken at heel/stolon end
☐	Peel taken at Rose end
☐	Plug taken at rose end

24.1 If ELISA is used in the laboratory for CMS how was it developed? (Same changes as for blackleg pathogens, add IF)

☐	In-house
☐	Commercial Kit
☐	Other
☐	Not used

24.2 If an in-house method is used, are you willing to share the method

☐	Yes
☐	No

If yes, contact

24.3 Commercial kit method.

☐	Yes
☐	No

If yes, please specify supplier.

24.4 Other ELISA Method (If yes, fill in blank)

25.1 If IF is used in the laboratory for CMS, how was it developed?

☐	In-house
☐	Commercial Kit
☐	Not Used

25.2 If an in-house IF method is used, are you willing to share the method?

☐	Yes*
☐	No

*To receive information contact

25.3 If you use a commercial IF kit method, please indicate supplier, please specify name and source.

25.4 If other IF Method, please specify

26.1 If PCR is used in your lab, how was the method for nucleic acid extraction developed?

☐	In-house
☐	Commercial Kit
☐	Crude sample (no extraction)
☐	Other
☐	Not used

26.2 If an in-house nucleic acid extraction method is used, are you willing to share the protocol?

☐	Yes
☐	No

26.3 Do you use a commercial kit method for nucleic acid extraction?

☐	Yes
☐	No

If yes, please specify supplier.

26.4 Other nucleic acid extraction method (If yes, fill in blank)

26.5 Are the tubers/stems pooled/bulked for PCR testing?

☐	Yes
☐	No

If Yes to the above question, what is the total number in each subsample?

Subsample size
☐50 ☐100 ☐200 ☐400

26.6 Are the PCR primer sequences published?

☐	Yes
☐	No

If yes, please provide references for the primer sequences

26.7. If primer sequences are not published, would the laboratory be willing to share sequences and protocols

☐	Yes
☐	No

27 If selective media is used to isolate CMS:

Please provide selective media information for CMS.

Questions on *Ralstonia solanacearum*, the Causal Agent of Brown Rot

28 Is *Ralstonia solanacearum* (Brown Rot) known to occur in your country:

☐	Yes
☐	No

29 Testing for *Ralstonia* species complex associated with brown rot in your country is:

☐	Compulsory for all crops as part of seed potato certification
☐	Compulsory for all crops with exemptions* under certain conditions
☐	Voluntary by grower
☐	For confirmation of visual symptoms
☐	Surveillance (do above for blackleg)
☐	Not done

30 *Ralstonia solanacearum* testing is conducted according to the following criteria – check all that apply

☐	Origin of seed
☐	Variety
☐	Crop Rotation
☐	Irrigation
☐	Customer request
☐	Inspection history/findings
☐	Surveillance
☐	Symptomatic plants

31 *Ralstonia solanacearum* testing is done by:

☐	Your organization
☐	Other governmental laboratory
☐	University or research institute
☐	Private laboratory
☐	Laboratory in other country
☐	Laboratory approved by the CA

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

☐	The reliability of tests
☐	The rapidity of tests
☐	The price of the tests
☐	Third party accreditation
☐	No possibility to choose
☐	Mandatory requirement to use a particular lab

33 Type of potato tissue tested for *Ralstonia solanacearum* (tick all that apply)

☐	Microplants
☐	Stems during growing season
☐	Tubers
☐	Both stems and tubers

34.1 Is enrichment used prior to conducting specific tests for *Ralstonia solanacearum*:

☐	Yes (see 34.3)
☐	No (see 34.4)

34.2 Is incubation of tubers at controlled temperature and humidity used to enhance populations prior to conducting specific tests on tubers?

☐	Yes (see 34.3)
☐	No (see 34.4)

34.3 What methods are used for testing for *Ralstonia solanacearum* (with enrichment or incubation)?

	Type of potato tissue tested		
	Microplants	Stems	Tubers
Pathogen	Method		
Ralstonia solani	o Not Tested o PCR o ELISA o IF o Selective Media o Other	o Not Tested o PCR o ELISA o IF o Selective Media o Other	o Not Tested o PCR o ELISA o IF o Selective Media o Other

34.4 What methods are used for testing for *Ralstonia solanacearum* (without enrichment or incubation)?

	Type of potato tissue tested		
	Microplants	Stems	Tubers
Pathogen	Method		
Ralstonia solani	o Not Tested o PCR o ELISA o IF o Selective Media o Other	o Not Tested o PCR o ELISA o IF o Selective Media o Other	o Not Tested o PCR o ELISA o IF o Selective Media o Other

34.5 Please specify the sample size required for microplants, stems and tubers when testing for *Ralstonia solanacearum*

	microplants	Stems	Tubers
Ralstonia solani	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600	☐1-50 ☐51-200 ☐201-400 ☐>400 ☐4600

34.6 What is the subsample size for analysis of the samples in question 30.5

	Subsample size
Ralstonia solani	☐50 ☐100 ☐200 ☐400

35 If tuber testing is conducted, what part of the tuber is sampled

☐	Peel taken at heel/stolon end
☐	Plug taken at heel/stolon end
☐	Both plug and peel
☐	Peel taken at Rose end
☐	Plug taken at rose end

36.1 If ELISA is used in the laboratory for *Ralstonia solanacearum*, how was it developed?

☐	In-house
☐	Commercial Kit
☐	Other
☐	Not used

36.2 If an in-house method is used, are you willing to share the method

☐	Yes
☐	No

Contact information

36.3 Commercial kit method.

☐	Yes
☐	No

If yes, please specify supplier.

36.4 Other ELISA Method (If yes, fill in blank)

37.1 If IF is used in the laboratory for Ralstonia, how was it developed?

☐	In-house
☐	Commercial Kit
☐	Not Used

37.2 If an in-house IF method is used, are you willing to share the method?

☐	Yes*
☐	No

Contact information

37.3 If you use a commercial IF kit method, please indicate supplier, please specify name and source.

37.4 If other IF Method, please specify

38.1 If PCR is used in the laboratory for *Ralstonia solanacearum* how was it developed?

☐	In-house
☐	Commercial Kit
☐	Crude sample (no extraction)
☐	Other
☐	Not used

38.2 If an in-house PCR method is used, are you willing to share the method

☐	Yes
☐	No

38.3 Commercial kit method for PCR

☐	Yes
☐	No

If yes, please specify supplier.

38.4 Other PCR Method (If yes, fill in blank)

38.5 Are the tubers/stems pooled/bulked for PCR testing?

☐	Yes
☐	No

If Yes to the above question, what is the total number of subsamples for

Stem samples:

Tuber samples:

38.6 Are the PCR primer sequences published?

☐	Yes
☐	No

Please provide references for the primer sequences

38.7 If primer sequences are not published, would laboratory be willing to share sequences and protocols?

☐	Yes
☐	No

39 Is sequencing used to determine speciation? (Is sequencing used to determine biovars)?

☐	Yes
☐	No

40 If selective media is used to isolate *Ralsonia*:

Please provide selective media information for *Ralstonia*.

41 How does the authority use the lab result? Check only those that apply

	Certification	Grower Information	Surveillance	Zero tolerance
Pectobacterium spp.	☐	☐	☐	☐
Dickeya solani	☐	☐	☐	☐
Other *Dickeya spp.*	☐	☐	☐	☐
Ralstonia solanacearum	☐	☐	☐	☐
Clavibacter michiganensis subsp. sepedonicus	☐	☐	☐	☐

Additional explanation

Quality Assurance

42 Is the laboratory accredited/approved for the above tests?

	Yes	No	NA
Pectobacterium spp.	☐	☐	☐
Dickeya solani	☐	☐	☐
Other *Dickeya spp.*	☐	☐	☐
Ralstonia solanacearum	☐	☐	☐
Clavibacter michiganensis subsp. sepedonicus	☐	☐	☐

43 Has the laboratory validated their PCR bacterial pathogen testing method?

	Yes	No	NA
Pectobacterium spp.	☐	☐	☐
Dickeya solani	☐	☐	☐
Other *Dickeya spp.*	☐	☐	☐
Ralstonia solanacearum	☐	☐	☐
Clavibacter michiganensis subsp. sepedonicus	☐	☐	☐

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

	Yes	No	NA
Pectobacterium spp.	☐	☐	☐
Dickeya solani	☐	☐	☐
Other *Dickeya spp.*	☐	☐	☐
Ralstonia solanacearum	☐	☐	☐
Clavibacter michiganensis subsp. sepedonicus	☐	☐	☐

45 Does the laboratory participate in any ring tests/ proficiency tests of potato bacterial pathogen testing by PCR?

	Yes	No	NA
Pectobacterium spp.	☐	☐	☐
Dickeya solani	☐	☐	☐
Other *Dickeya spp.*	☐	☐	☐
Ralstonia solanacearum	☐	☐	☐
Clavibacter michiganensis subsp. sepedonicus	☐	☐	☐

46 Does the seed potato certification authority audit the laboratory and testing procedures?

	Yes	No	NA
Pectobacterium spp.	☐	☐	☐
Dickeya solani	☐	☐	☐
Other *Dickeya spp.*	☐	☐	☐
Ralstonia solanacearum	☐	☐	☐
Clavibacter michiganensis subsp. sepedonicus	☐	☐	☐

47 Does the laboratory have an internal Quality Control system?

☐	Yes
☐	No

* *** *