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Specialized Section on Standardization of Seed Potatoes
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Item 5 of the provisional agenda
Draft survey on bacterial testing methodologies

Revised survey on bacterial testing methodologies *

Submitted by the secretariat

The following document was prepared by the delegation from the United States on behalf of the working group. The comments of the working group and the rapporteur have been integrated into this new draft. The Specialized Section is invited to review the draft survey in view of its approval.

This document is submitted according to ECE/CTCS/2017/10 section II c, ECE/CTCS/2018/2 section VII a, and A/74/6 (Sect.20), para 20.37; and Supplementary.

* Submitted on the above date to include all comments from the working group.

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

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

i.	☐	Yes
ii.	☐	No

If yes, specify which Pectobacteria are known to occur:

i.	☐	Pectobacterium atrosepticum
ii.	☐	Pectobacterium carotovorum subsp. carotovorum
iii.	☐	Pectobacterium carotovorum subsp. brasiliense
iv.	☐	Pectobacterium carotovorum subsp. odorifera
v.	☐	Pectobacterium wasabiae
vi.	☐	Pectobacterium parmentieri
vii.	☐	Other
viii.	☐	Do not know species

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

i.	☐	Yes
ii.	☐	No

If yes, what Dickeya species are known to occur?

i.	☐	*Dickeya dianthicola*
ii.	☐	*Dickeya solani*
iii.	☐	*Dickeya zea*
iv.	☐	Other
v.	☐	Do not know species

4. Potato blackleg laboratory testing in your country is:

	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
	☐	☐	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 (Dickeya and Pecto columns)

	Pectobacterium spp.	*Dickeya* spp.	
i.	☐	☐	Origin of seed
ii.	☐	☐	Variety
	☐	☐	By Class
iii.	☐	☐	Crop Rotation
iv.	☐	☐	Irrigation source
v.	☐	☐	Customer request
vi.	☐	☐	Inspection history/findings
vii	☐	☐	Surveillance
vii	☐	☐	Symptomatic plants

6. Blackleg testing is done by: (Should we divide this into columns too?)

i.	☐	Your organization
ii.	☐	Other governmental laboratory
iii.	☐	University or research institute
iv.	☐	Private laboratory
v.	☐	Laboratory in other country
vi.	☐	Laboratory approved by the CA

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

i.	☐	The reliability of tests
ii.	☐	The rapidity of tests
iii.	☐	The price of the tests
iv.	☐	Third party accreditation
v.	☐	No possibility to choose
vi.	☐	Mandatory requirement to use a particular lab

8. Type of potato tissue tested for blackleg pathogens (tick all that apply)

i.	☐	Microplants
ii.	☐	Stems during growing season
iii.	☐	Tubers

9. Is enrichment used prior to conducting specific tests?

i.	☐	Yes (see 9.1)
ii.	☐	No (see 9.2)

9.1 What methods are used for testing for pathogens causing blackleg disease (with enrichment)?

	Type of potato tissue tested		
	Microplants	Stems	Tubers
Pathogen	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

9.2 What methods are used for testing for pathogens causing blackleg disease (without enrichment)?

	Type of potato tissue tested		
	Microplants	Stems	Tubers
Pathogen	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

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

i.	☐	Yes
ii.	☐	No

9.4. 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 ☐50-200 ☐200-400 ☐>400 ☐4600*	☐1-50 ☐50-200 ☐200-400 ☐>400 ☐4600	☐1-50 ☐50-200 ☐200-400 ☐>400 ☐4600

*reference

9.5 What is the subsample size for analysis of the samples in question 9.4?

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

10. If tuber testing is conducted, what part of the tuber is sampled? (Tick all that apply.)

i.	☐	Peel taken at heel/stolon end
ii.	☐	Plug taken at heel/stolon end
iii.	☐	Peel taken at Rose end
iv.	☐	Plug taken at rose end

11. If ELISA is used in the laboratory, how was it developed? (if not used go to question 9)

i.	☐	In-house
ii.	☐	Commercial Kit
iii.	☐	Other

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

i.	☐	Yes*
ii.	☐	No

*To receive information contact

11.2 Commercial ELISA kit method.

i.	☐	Yes
ii.	☐	No

If yes, please specify supplier.

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

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

iv.	☐	In-house
v.	☐	Commercial Kit
vi.	☐	Other

12.1 If PCR was developed in-house, what is the reference and/or contact information?

12.2 Commercial kit method for PCR.

i. ☐ Yes
ii. ☐ No

If yes, please specify supplier.

12.3 If your PCR assay utilizes a commercial kit, what is the kit name and supplier?

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

iii.	☐	Yes
iv.	☐	No

Please supply contact for method

12.5. Commercial kit method for nucleic acid extraction?

i.	☐	Yes
ii.	☐	No

If yes, please specify supplier.

12.6 Other nucleic acid extraction Method (If yes, fill in blank)

12.7 Are the stems/ tubers pooled/bulked for PCR testing? ☐ **Yes** ☐ **No**

13. 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 ☐50-200 ☐200-400 ☐>400 ☐4600*	☐1-50 ☐50-200 ☐200-400 ☐>400 ☐4600

*reference on testing 0 tolerance

13.1 What is the subsample size for analysis of the samples in question 13?

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

14. Are the PCR primer sequences published? ☐ **Yes** ☐ **No**

Please provide references for the primer sequences

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

i.	☐	Yes
ii.	☐	No

Contact for primer sequences and protocol

15. Is sequencing used to determine speciation? Put in columns for pecto and dickeya?

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

16. Is Clavibacter michiganensis subsp. sepedonicus (Bacterial Ring Rot) known to occur in your country?

i.	☐	Yes
ii.	☐	No

17. *Clavibacter michiganensis subsp. sepedonicus* testing in your country is

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

*Exemptions may include seed class, generation, variety

18. *Clavibacter michiganensis subsp. sepedonicus* testing is conducted according to the following criteria – check all that apply

i.	☐	Origin of seed
ii.	☐	Variety
iii.	☐	Class
iv.	☐	Crop Rotation
v.	☐	Irrigation source
vi.	☐	Customer request
vii	☐	Inspection history/findings
vii	☐	Surveillance
viii	☐	Symptomatic plants

19. *Clavibacter michiganensis subsp. sepedonicus* testing is done by:

i.	☐	Your organization
ii.	☐	Other governmental laboratory
iii.	☐	University or research institute
iv.	☐	Private laboratory
v.	☐	Laboratory in other country
vi.	☐	Laboratory approved by the CA

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

i.	☐	The reliability of tests
ii.	☐	The rapidity of tests
iii.	☐	The price of the tests
iv.	☐	Third party accreditation
v.	☐	No possibility to choose
vi.	☐	Mandatory requirement to use a particular lab

21. Type of potato tissue tested for *Clavibacter michiganensis subsp. sepedonicus* (tick all that apply)

i.	☐	Microplants
ii.	☐	Stems during growing season
iii.	☐	Tubers

21.1. Is enrichment used prior to conducting specific tests for *Clavibacter michiganensis subsp. sepedonicus*?

i.	☐	Yes (see 21.2)
ii.	☐	No (see 21.3)

21.2 Is incubation of tubers at controlled temperature and humidity used to enhance populations prior to conducting specific tests on tubers? (move up by enrichment question)

i.	☐	Yes
ii.	☐	No

21.3 What methods are used for testing for *Clavibacter michiganensis subsp. Sepedonicus?*

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

21.4. Please specify the sample size required for microplants, stems and tubers for BRR

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

21.5 What is the subsample size for analysis of the samples in question 18.4

	Subsample size
Clavibacter michiganensis subsp. sepedonicus	☐50 ☐100 ☐200 ☐400

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

i.	☐	Peel taken at heel/stolon end
ii.	☐	Plug taken at heel/stolon end
iii.	☐	Peel taken at Rose end
iv.	☐	Plug taken at rose end

23. If ELISA is used in the laboratory for *Clavibacter michiganensis subsp. sepedonicus* how was it developed? (Same changes as for blackleg pathogens, add IF)

i.	☐	In-house
ii.	☐	Commercial Kit
iii.	☐	Other
iv.	☐	Not used

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

v.	☐	Yes
vi.	☐	No

23.2**.** Commercial kit method.

i.	☐	Yes
ii.	☐	No

If yes, please specify supplier.

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

24. If nucleic acid extraction is used in the laboratory for Clavibacter michiganensis subsp. sepedonicus how was it developed? (change to same as for blackleg extraction

v.	☐	In-house
vi.	☐	Commercial Kit
vii.		Crude sample (no extraction)
viii.	☐	Other
ix.	☐	Not used

24.1 If an in-house PCR method is used, are you willing to share the method (as above)?

vii.	☐	Yes
viii.	☐	No

24.2. Commercial kit method for PCR (as above)

i.	☐	Yes
ii.	☐	No

If yes, please specify supplier.

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

25. Are the tubers/stems pooled/bulked for PCR testing? ☐ Yes ☐ No

If yes, what is the total number of subsamples for: (Add tables for sample sizes back in.)

26. Are the PCR primer sequences published? ☐ Yes ☐ No

Please provide references for the primer sequences

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

i.	☐	Yes
ii.	☐	No

27. Is sequencing used to determine speciation?

i.	☐	Yes
ii.	☐	No

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

i.	☐	Yes
ii.	☐	No

29. Testing for *Ralstonia* species complex associated with brown rot in your country is? (make same changes as for Clavibacter)

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

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

i.	☐	Origin of seed
ii.	☐	Variety
iii.	☐	Crop Rotation
iv.	☐	Irrigation
v.	☐	Customer request
vi.	☐	Inspection history/findings
vii	☐	Surveillance
vii	☐	Symptomatic plants

31. *Ralstonia solanacearum* testing is done by:

vii.	☐	Your organization
viii.	☐	Other governmental laboratory
ix.	☐	University or research institute
x.	☐	Private laboratory
xi.	☐	Laboratory in other country
xii.	☐	Laboratory approved by the CA

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

vii.	☐	The reliability of tests
viii.	☐	The rapidity of tests
ix.	☐	The price of the tests
x.	☐	Third party accreditation
xi.	☐	No possibility to choose
xii.	☐	Mandatory requirement to use a particular lab

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

iv.	☐	Microplants
v.	☐	Stems during growing season
vi.	☐	Tubers
vii.	☐	Both stems and tubers

33.1. Is enrichment used prior to conducting specific tests for *Ralstonia solanacearum*:?

i.	☐	Yes (see 6.2)
ii.	☐	No (see 6.3)

33.2. What methods are used for testing for *Ralstonia solanacearum* (with enrichment)?

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

33.3 What methods are used for testing for Ralstonia solanacearum (without enrichment)?

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

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

i.	☐	Yes
ii.	☐	No

33.5 Method used for postharvest bacterial pathogen testing of tubers after incubation.

Pathogen	Method
Ralstonia solani	○ Not Tested ○ PCR ○ ELISA ○ Selective Media ○ Other

33.6. Please specify the sample size required for microplants, stems and tubers for *Ralstonia solanacearum*

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

33.7 What is the subsample size for analysis of the samples in question 18.6?

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

34. If tuber testing is conducted, what part of the tuber is sampled? (repeat under each group of pathogesn)

i.	☐	Peel taken at heel/stolon end
ii.	☐	Plug taken at heel/stolon end
iii.	☐	Both plug and peel
iv.	☐	Peel taken at Rose end
v.	☐	Plug taken at rose end

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

i.	☐	In-house
ii.	☐	Commercial Kit
iii.	☐	Other
iv.	☐	Not used

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

i.	☐	Yes
ii.	☐	No

35.2 Commercial kit method.

i.	☐	Yes
ii.	☐	No

If yes, please specify supplier.

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

36. If PCR is used in the laboratory for Ralstonia solanacearum how was it developed?

i.	☐	In-house
ii.	☐	Commercial Kit
iii.	☐	Other
iv.	☐	Not used

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

i.	☐	Yes
ii.	☐	No

36.2 Commercial kit method for PCR

i.	☐	Yes
ii.	☐	No

If yes, please specify supplier.

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

37. Are the tubers/stems pooled/bulked for PCR testing? ☐ **Yes** ☐ **No**

If yes, what is the total number of subsamples for

Stem samples:

Tuber samples:

38. Are the PCR primer sequences published? ☐ **Yes** ☐ **No**

Please provide references for the primer sequences

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

i.	☐	Yes
ii.	☐	No

39. Is sequencing used to determine speciation?

i.	☐	Yes
ii.	☐	No

40. How does the authority use the lab result? Tick 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

41. Is the laboratory accredited/approved for the above tests? (**Put in table with spp. And zero tolerance pathogens.)**

Yes ☐ **No** ☐

42. Does the laboratory have an internal Quality Control system?

☐ **Yes** ☐ **No**

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

☐ **Yes** ☐ **No** ☐ **In progress (Add in box for NA)**

43.1 Have the PCR methods used for certification been independently validated/accredited? According to pathogen

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

☐ **Yes** ☐ **No**

44. Does the seed potato certification authority audit the laboratory and testing procedures? (according to pathogen)

Laboratory: ☐ **Yes** ☐ **No**
Testing Procedures: ☐ **Yes** ☐ **No**