

Survey of Testing Methods for Bacterial Pathogens of Potato that are Associated with Seed Certification

Specialized Section on Standardization of
Seed Potatoes



Survey Respondents



59 Total Responses

The survey covered testing methods for bacterial pathogens of potato that are associated with seed certification. There were 59 responses received from 32 countries. 34 of the responses were complete, and 25 were partially complete. More than one response was received from multiple countries.

Participating countries:

- ✓ Australia
- ✓ Belgium
- ✓ Bulgaria
- ✓ Croatia
- ✓ Cyprus
- ✓ Czech Republic
- ✓ Denmark
- ✓ Egypt
- ✓ Estonia
- ✓ Finland
- ✓ France
- ✓ Germany
- ✓ Greece
- ✓ Italy
- ✓ Japan
- ✓ Latvia
- ✓ Lithuania
- ✓ Luxembourg
- ✓ New Zealand
- ✓ The Netherlands
- ✓ Poland
- ✓ Republic of Ireland
- ✓ Russian Federation
- ✓ Serbia
- ✓ Slovak Republic
- ✓ Slovenia
- ✓ South Africa
- ✓ Sweden
- ✓ Switzerland
- ✓ United Kingdom
- ✓ United States



Pathogens

The survey asked a series of questions about the following pathogens:

- ✓ Individual blackleg pathogens within *Pectobacterium* spp. and *Dickeya* spp.
- ✓ *Clavibacter michiganensis* subsp. *sepedonicus* (CMS)
- ✓ *Ralstonia solanacearum* (Brown Rot)

Question Topics

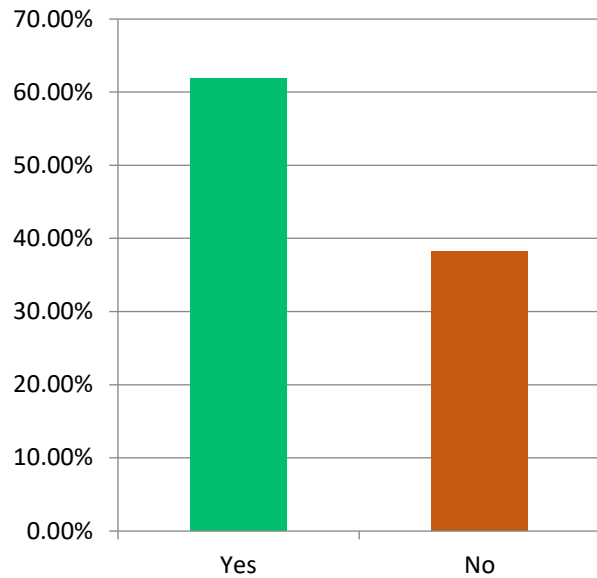
- ✓ Background questions
- ✓ Legal requirements
- ✓ Testing methodologies
- ✓ Testing criteria
- ✓ Quality control

Testing Background Questions

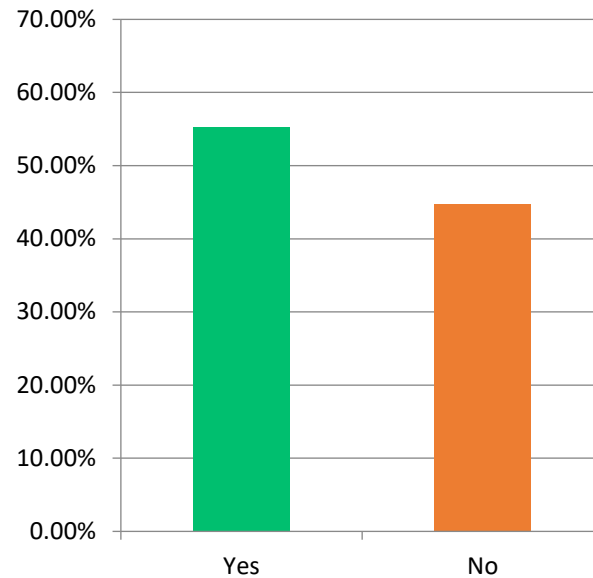


Is the disease or pathogen a problem in your country/state?

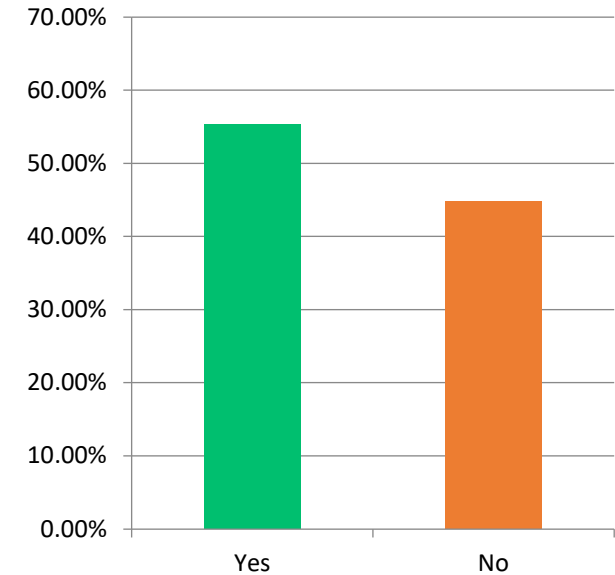
Blackleg



CMS



Ralstonia



Testing Background Questions



Is testing compulsory?

Blackleg

	Pectobacterium spp.		Dickeya spp.	
Compulsory for all crops as part of seed potato certification	8.51%	4	8.51%	4
Compulsory for all crops with exemptions under certain conditions	2.13%	1	6.38%	3
Voluntary by grower	38.30%	18	36.17%	17
Compulsory if found in field	12.77%	6	12.77%	6
For confirmation of visual symptoms	53.19%	25	53.19%	25
Not done	29.79%	14	31.91%	15
			Answered	47
			Skipped	12

CMS

	Responses	
Compulsory for all crops as part of seed potato certification	62,16%	23
Compulsory for all crops with exemptions under certain conditions (exemptions may include seed class, generation, variety)	5,41%	2
Voluntary by grower	2,70%	1
For confirmation of visual symptoms	8,11%	3
Surveillance (do above for blackleg)	10,81%	4
Not done	10,81%	4
	Answered	37
	Skipped	22

Ralstonia solanacearum

	Answer Choices		Responses	
Compulsory for all crops as part of seed potato certification	69.44%	25		
Compulsory for all crops with exemptions under certain conditions	0.00%	0		
Voluntary by grower	2.78%	1		
For confirmation of visual symptoms	11.11%	4		
Surveillance (do above for blackleg)	8.33%	3		
Not done	8.33%	3		
			Answered	36
			Skipped	23

Testing Methodology



- ✓ The following questions cover the testing methodology, including
 - ✓ Sample size
 - ✓ Part of the plant used to test
 - ✓ Technologies used
- ✓ Examples from the following slides use data from questions on **Blackleg**, but responses to these questions were also collected for CMS and Ralstonia

Testing Methodology



Testing is conducted according to the following criteria:

	Pectobacterium spp.		Dickeya spp.	
Origin of seed	20.00%	8	22.50%	9
Variety	5.00%	2	5.00%	2
By Class	12.50%	5	12.50%	5
Crop Rotation	2.50%	1	2.50%	1
Irrigation source	2.50%	1	5.00%	2
Customer request	57.50%	23	57.50%	23
Inspection history/findings	22.50%	9	17.50%	7
Surveillance	25.00%	10	27.50%	11
Symptomatic plants	60.00%	24	60.00%	24
		Answered		40
		Skipped		19

Testing is done by:

Answer Choices	Responses	
Your organization	52.38%	22
Other governmental laboratory	19.05%	8
University or research institute	23.81%	10
Private laboratory	11.90%	5
Laboratory in other country	2.38%	1
Laboratory approved by the CA	19.05%	8
	Answered	42
	Skipped	17

Criteria to choose laboratory:

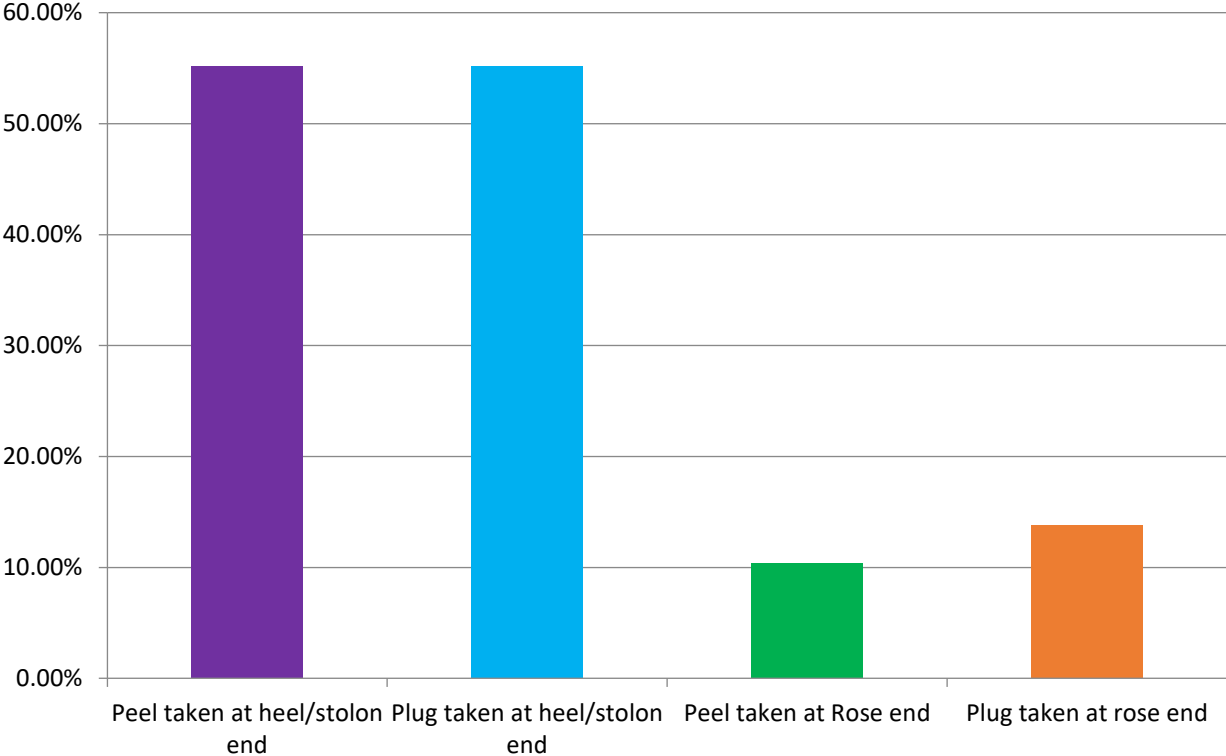
Answer Choices	Responses	
The reliability of tests	57.50%	23
The rapidity of tests	45.00%	18
The price of the tests	27.50%	11
Third party accreditation	25.00%	10
No possibility to choose	15.00%	6
Mandatory requirement to use a particular lab	22.50%	9
	Answered	40
	Skipped	19

Figures shown are for Blackleg

Testing Methodology



What part of the tuber is sampled?



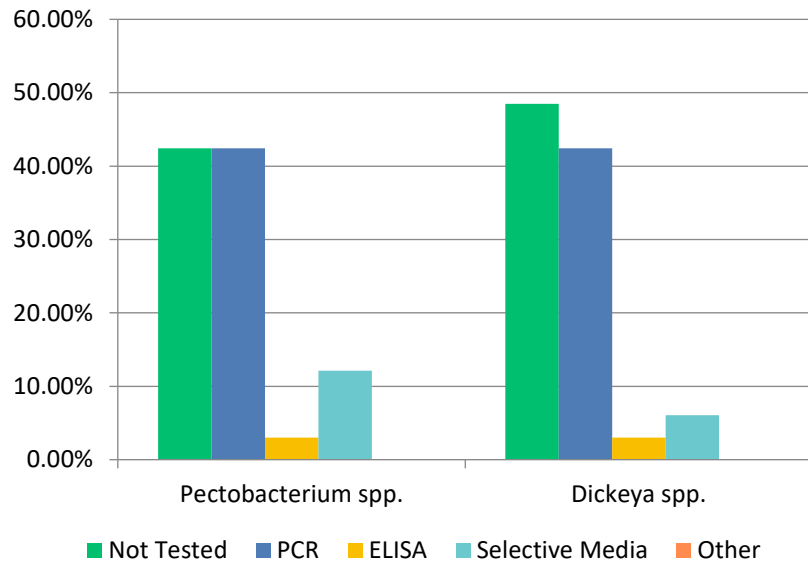
Figures shown are for Blackleg

Testing Methodology

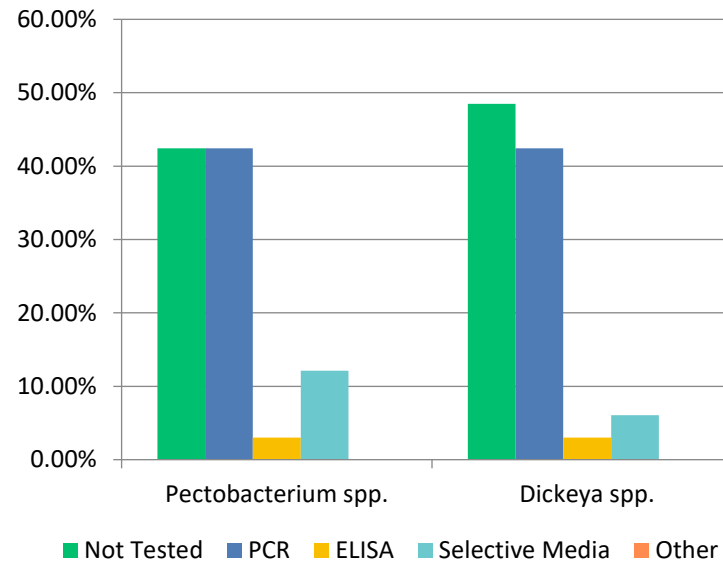


What methods are used for testing for pathogens?

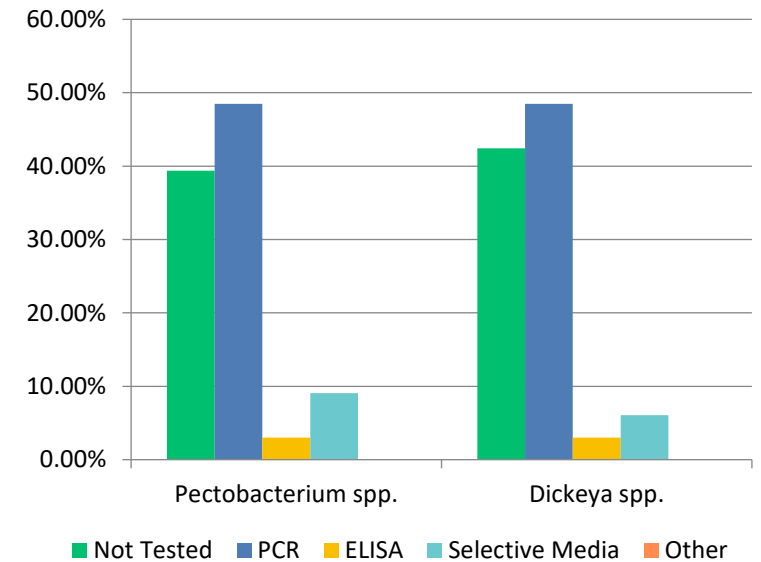
Microplants



Stems



Tubers

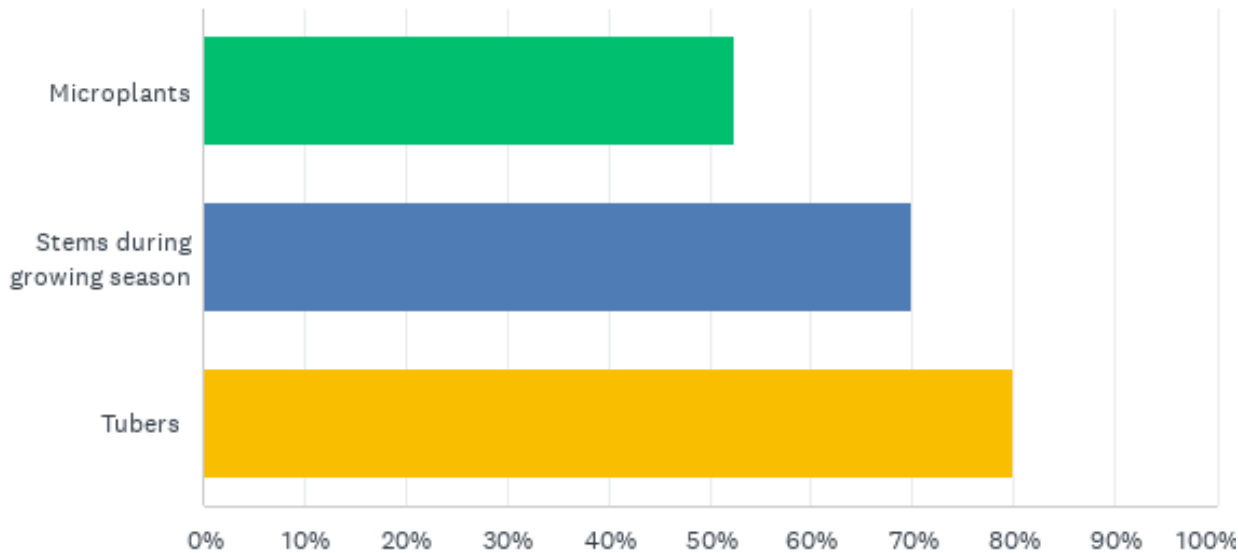


Figures shown here are for Blackleg disease **with** enrichment or incubation (question was also asked for samples without enrichment or incubation)

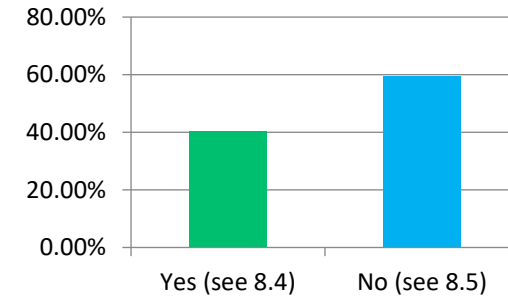
Testing Methodology



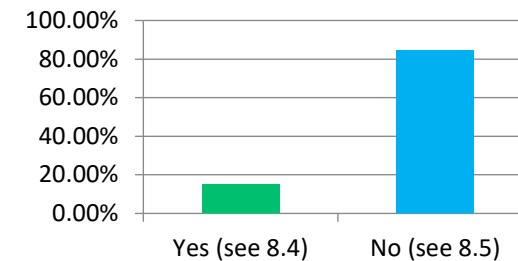
Type of potato tissue tested:



Is enrichment used prior to conducting specific tests:



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



Figures shown are for Blackleg

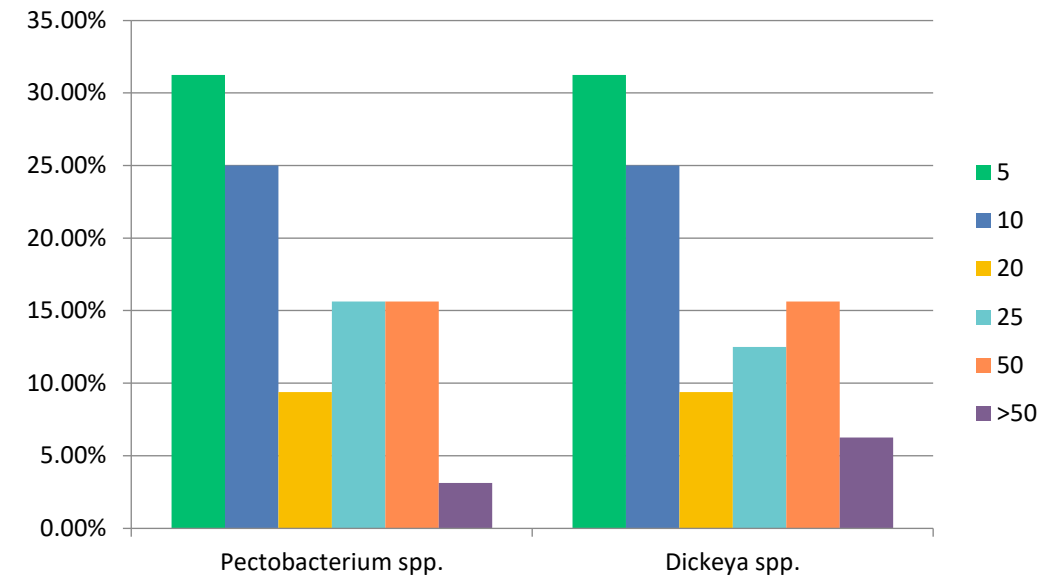
Testing Methodology: PCR



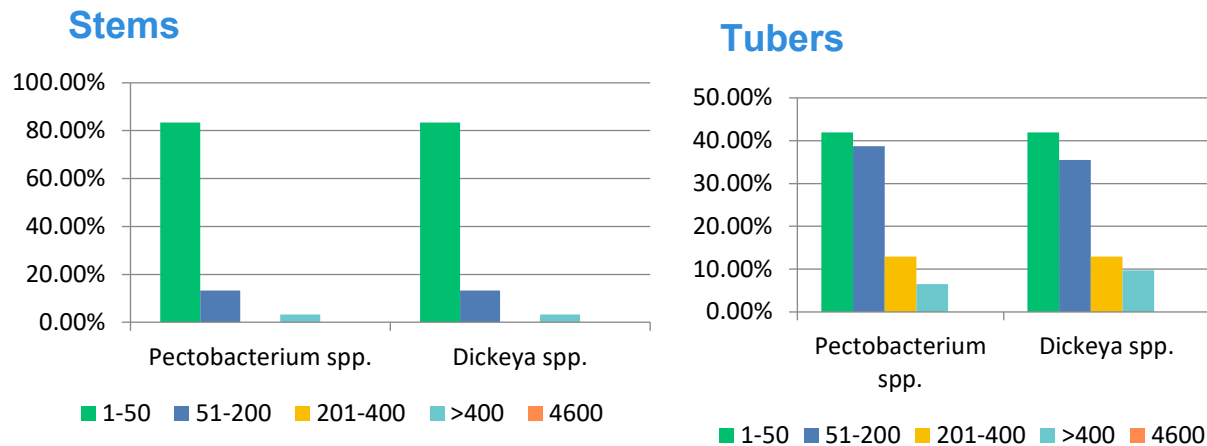
Are the stems/ tubers pooled/bulked for PCR testing?

Answer Choices	Responses	
Yes	75,00%	21
No	25,00%	7
Answered		28
Skipped		31

What is the subsample size for PCR testing?



Sample size required for stems and tubers in PCR testing:

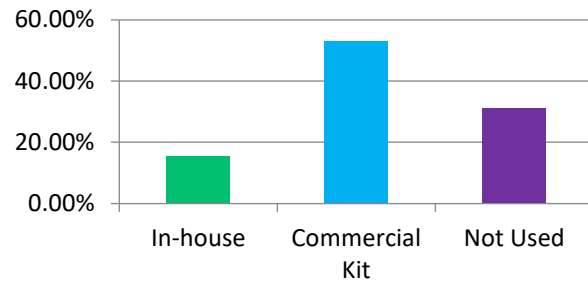


Figures shown are for Blackleg

Testing Methodology: PCR



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



Reference and/or contact information if protocol was developed in house?

- ✓ R. Czajkowski et al. Detection, Identification and differentiation of Pectobacterium and Dickeya species causing potato blackleg and tuber soft rot.
- ✓ Pritchard et al. (2012). Detection of phytopathogens of the genus Dickeya using a PCR primer prediction pipeline for draft bacterial genome sequences.

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

Answer Choices	Responses	Count
Yes	12,50%	2
No	81,25%	13
Answered		16
Skipped		43

Commercial kit name and supplier, if used

- ✓ Qiagen Biosprint 15 DNA Plantkit
- ✓ BIOREBA AG Christoph Merian-Ring 7
- ✓ NucleoSpin Tissue, TaKaRa
- ✓ Bioline, Qiagen
- ✓ Agdia, RPA
- ✓ Quagen proplant

Other nucleic acid extraction method

- ✓ CTAB
- ✓ Enrichment culturing prior to the PCR
- ✓ Boiling of suspensions of bacterial isolates for 2 min and then chilling them on ice for 5 min

Testing Methodology: PCR



Are the PCR primer sequences published?

Answer Choices	Responses	
Yes	48,15%	13
No	29,63%	8

References for primer sequences

- ✓ Nassar ADE1/ADE2
- ✓ Czajkowski et al. (2015) Annals of Applied Biology, 166: 18-38
- ✓ Humphris et al, 2015. Methods in Molecular Biology

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

Answer Choices	Responses	
Yes	22,22%	4
No	61,11%	11

Testing Methodology: ELISA



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

Answer Choices	Responses	
In-house	0,00%	0
Commercial Kit	11,11%	4
Not Used	88,89%	32
	Answered	36
	Skipped	23

Do you use a commercial ELISA kit method?

Answer Choices	Responses	
Yes	12,50%	3
No	83,33%	20
	Answered	24
	Skipped	35

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

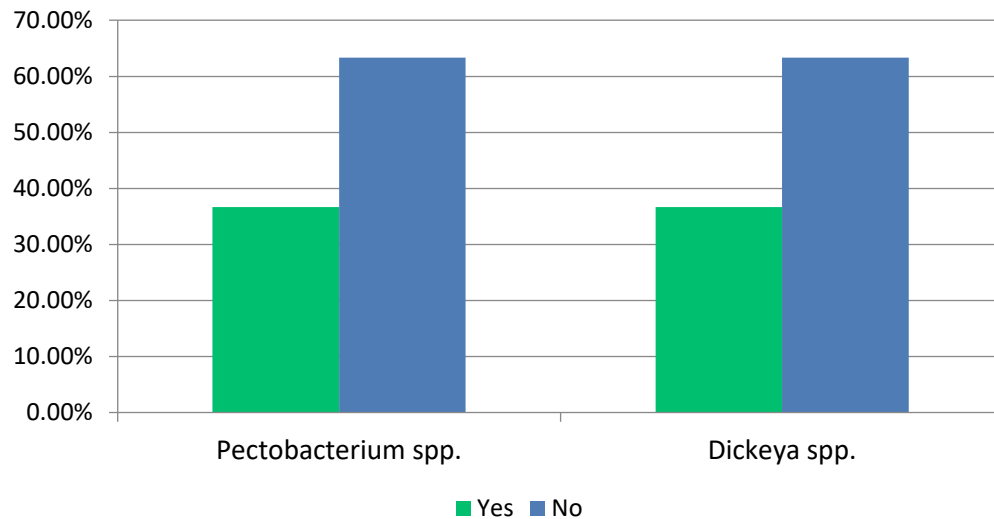
Answer Choices	Responses	
Yes	5,88%	1
No	94,12%	16
	Answered	17
	Skipped	42

No other ELISA methods reported

Testing Methodology



Is sequencing used to identify the species?



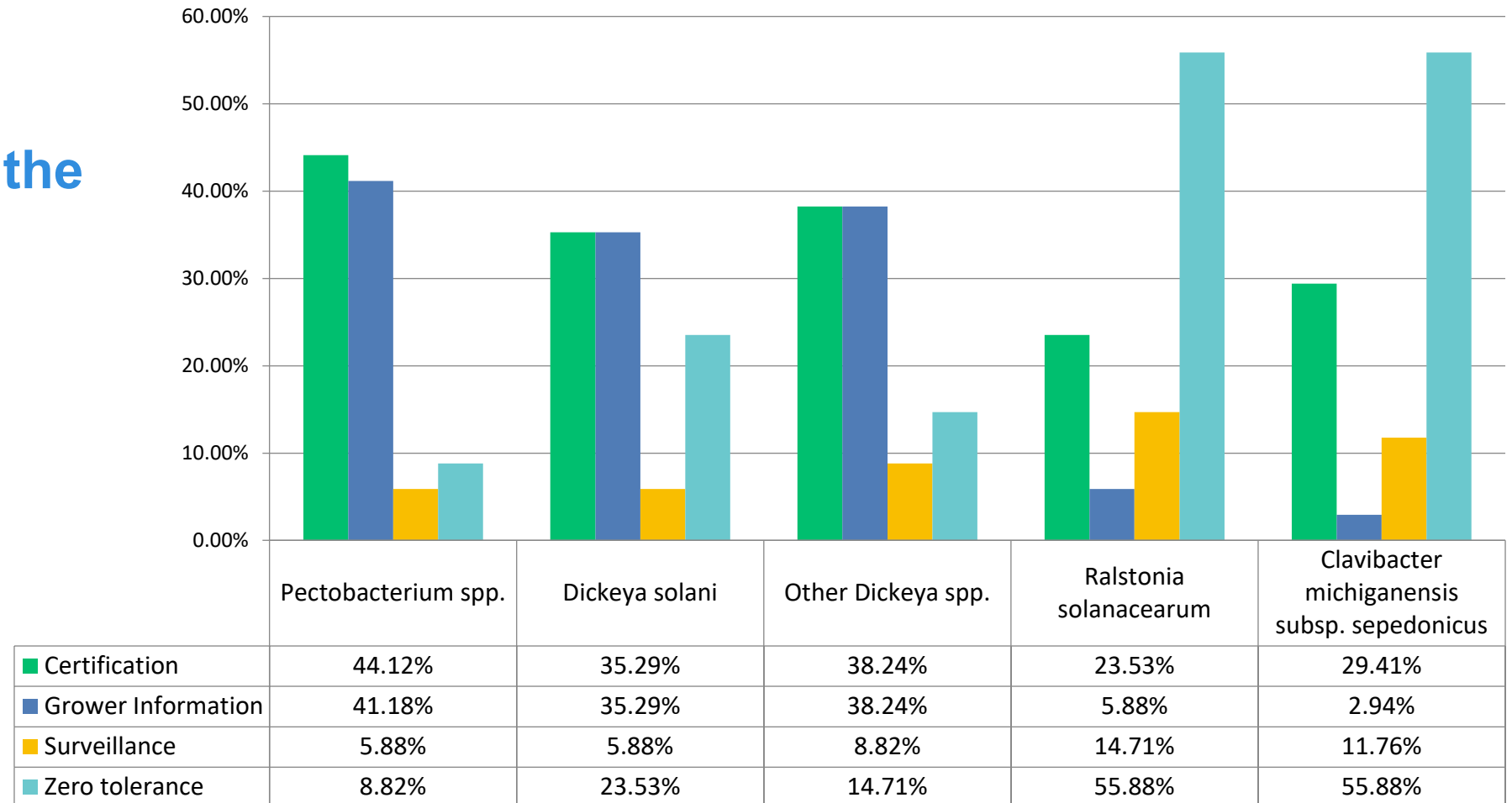
If selective media is used, please provide selective media information

- ✓ Crystal Violet Pectate (CVP)
- ✓ King's B
- ✓ Pectate Enrichment Medium (PEM)

Use of Lab Results



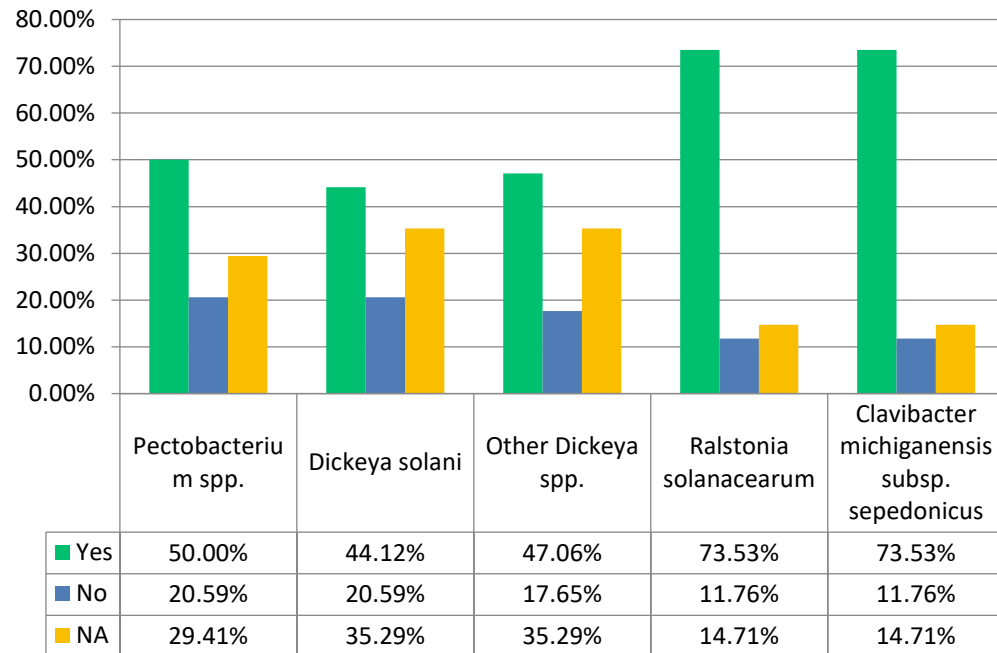
How does the authority use the lab result?



Quality Control

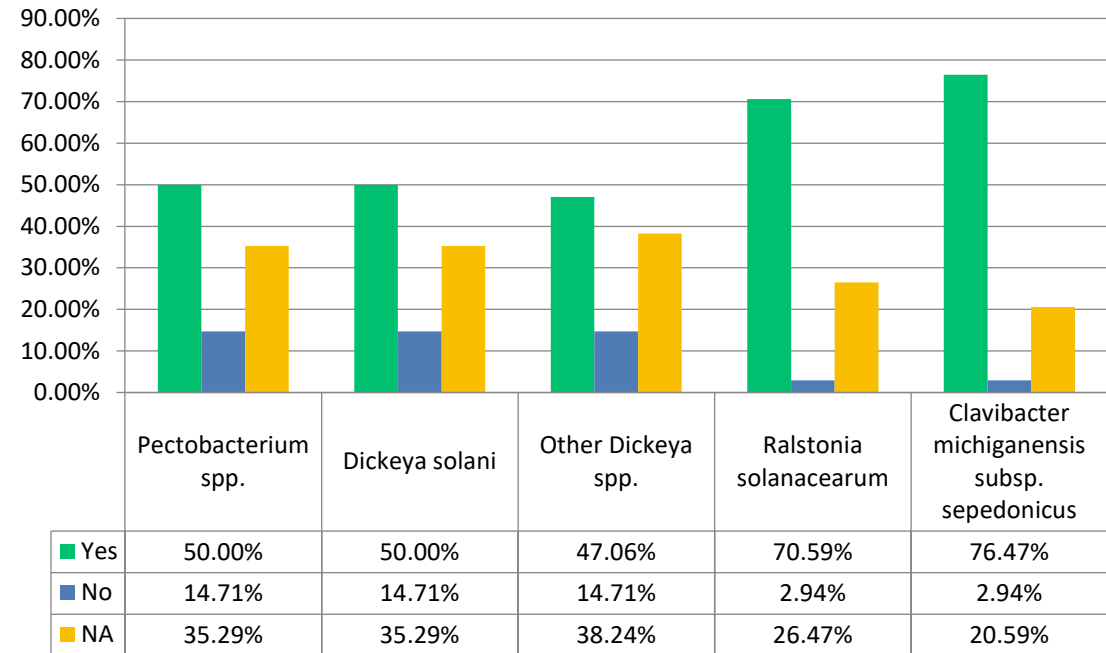


Is the laboratory accredited/approved for the above tests?



Answered	34
Skipped	25

Has the laboratory validated their PCR bacterial pathogen testing method?

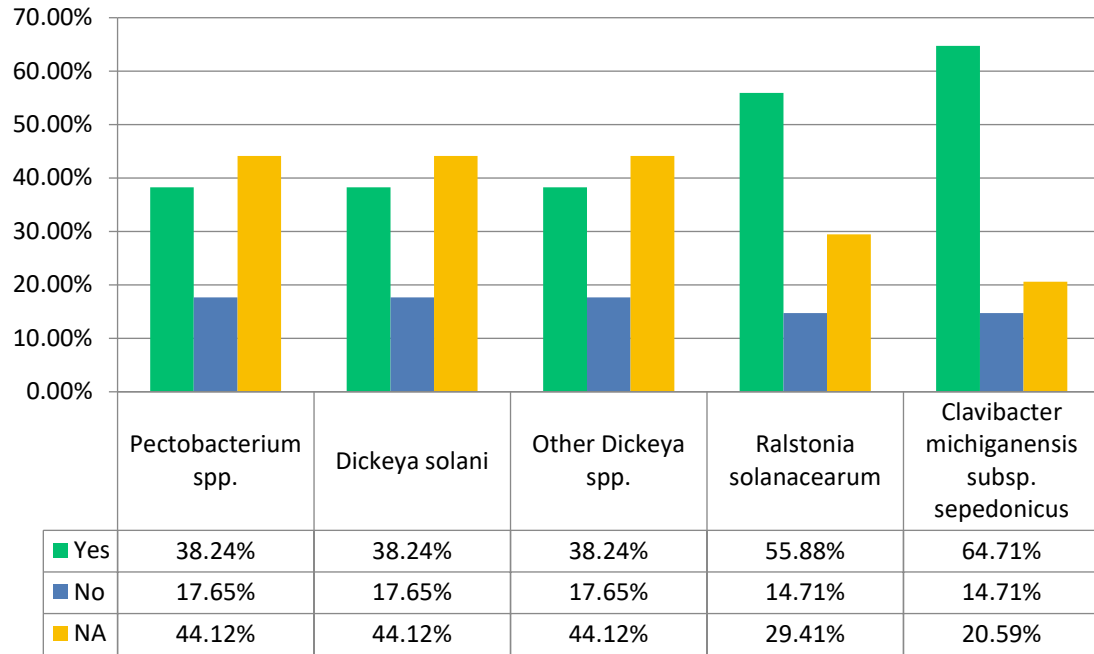


Answered	34
Skipped	25

Quality Control

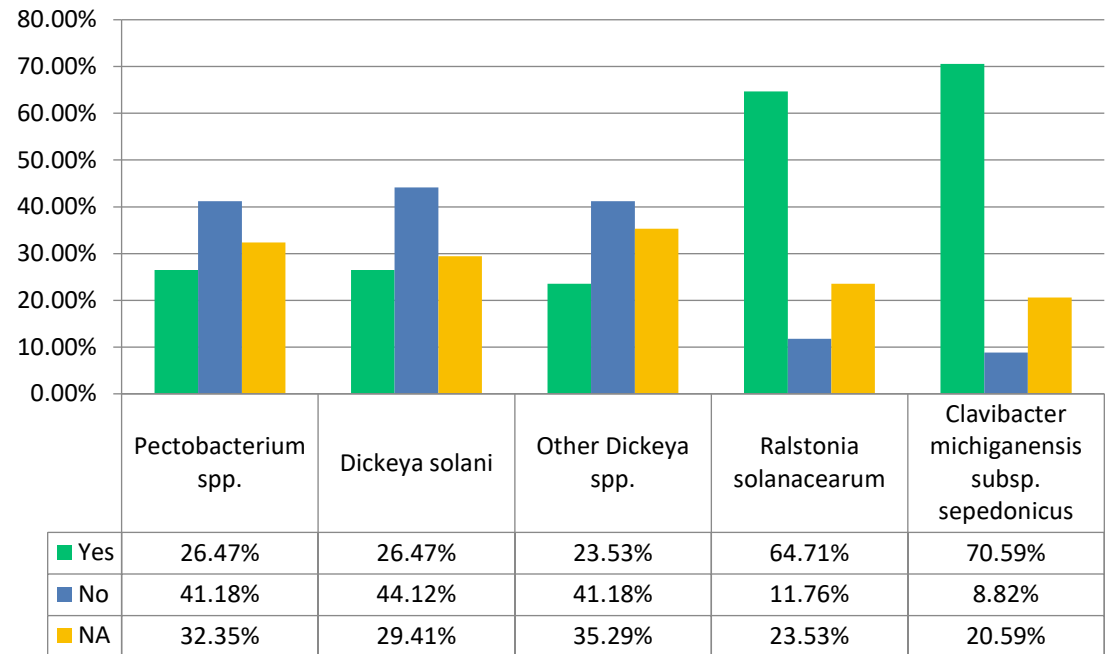


Have the PCR methods used for certification been independently validated/accredited?



Answered	34
Skipped	25

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



Answered	34
Skipped	25

Quality Control



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

	Yes	No	NA	Total
Pectobacterium spp.	23.53% 8	50.00% 17	26.47% 9	34
Dickeya solani	23.53% 8	47.06% 16	29.41% 10	34
Other Dickeya spp.	23.53% 8	47.06% 16	29.41% 10	34
Ralstonia solanacearum	32.35% 11	44.12% 15	23.53% 8	34
Clavibacter michiganensis subsp. sepedonicus	38.24% 13	44.12% 15	17.65% 6	34
			Answered	34
			Skipped	25

Does the laboratory have an internal Quality Control system?

Answer Choices	Responses
Yes	88.24% 30
No	11.76% 4
	Answered 34
	Skipped 25

Summary of Results



Key Findings:

- ✓ Most questions had between 30 and 40 responses
- ✓ Open ended questions had a lower response rate, particularly the ones regarding testing methods used (ELISA, PCR, IF)
- ✓ Blackleg had the highest proportion of respondents reporting it as a problem, and questions relating to it had the highest response rates
- ✓ CMS and *Ralstonia* had similar responses for quality control and testing methodology questions

Thank you!

