Findings of the survey of Seed Potato Virus Testing Methods associated with Seed Potato Certification

Compiled by the UNECE secretariat, 20 December 2017, updated 2019

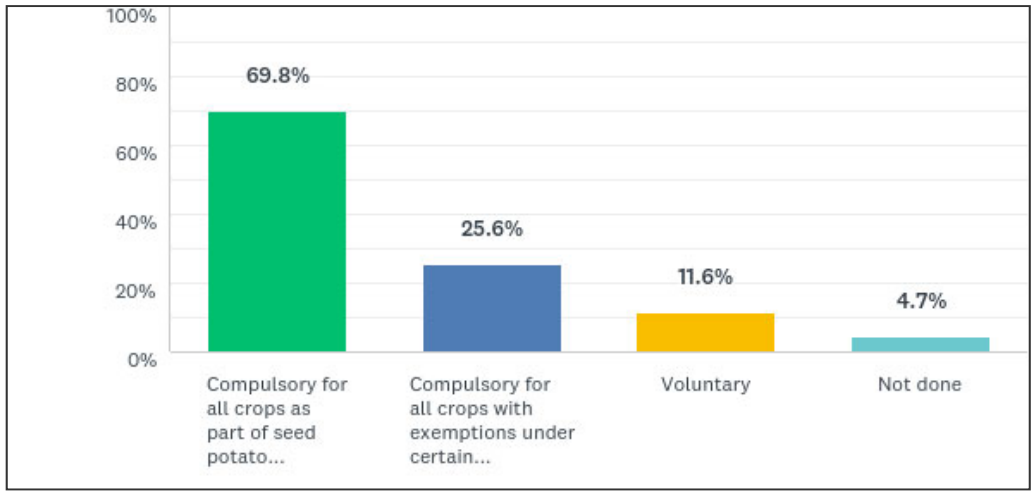
45 Total Responses

Certification Authorities from 38 countries (including Malta and Portugal*) responded to the survey. The questionnaire was sent to 43 countries resulting the answering percentage 88%. More than one response was received from several countries.

- ✓ USA (Oregon, Wisconsin, Montana, North Dakota, Colorado, Alaska)
- ✓ Cyprus
- ✓ Greece
- ✓ Iceland
- ✓ UK (England/Wales, Scotland, N. Ireland)
- ✓ Hungary
- ✓ The Netherlands
- ✓ Finland
- ✓ Sweden
- ✓ Lithuania
- ✓ Belgium
- ✓ Czech Republic
- ✓ Germany
- ✓ Norway
- ✓ Canada
- ✓ Bulgaria
- ✓ Egypt
- ✓ Latvia
- ✓ Poland
- ✓ Slovak Republic
- ✓ Estonia
- ✓ Spain (Castilla Y Leon, Pais Vasco)
- ✓ Switzerland
- ✓ New Zealand
- ✓ South Africa
- ✓ Austria
- ✓ France
- ✓ India
- ✓ China
- ✓ Italy
- ✓ Slovenia
- ✓ Denmark
- ✓ Australia
- ✓ Luxembourg
- ✓ Russian Federation
- ✓ Chile

* Malta and Portugal, no seed potato certification schema

1. Potato virus testing in your country is

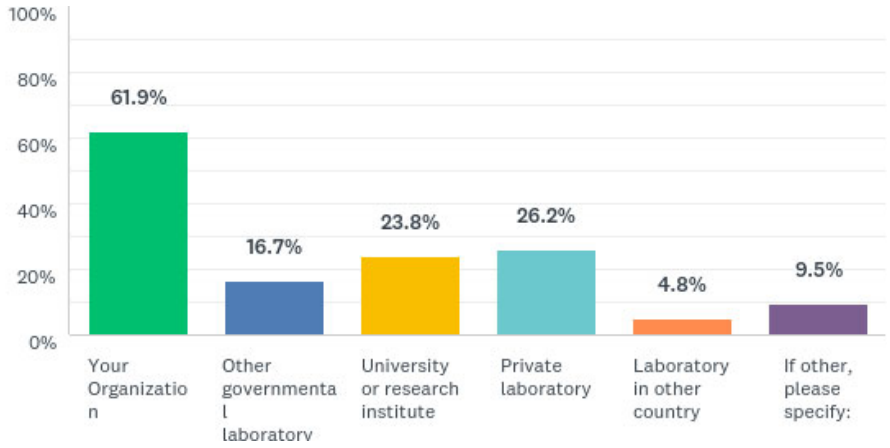


ANSWER CHOICES	RESPONSES	
Compulsory for all crops as part of seed potato certification	69.8%	30
Compulsory for all crops with exemptions under certain conditions	25.6%	11
Voluntary	11.6%	5
Not done	4.7%	2
Total Respondents: 43		

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

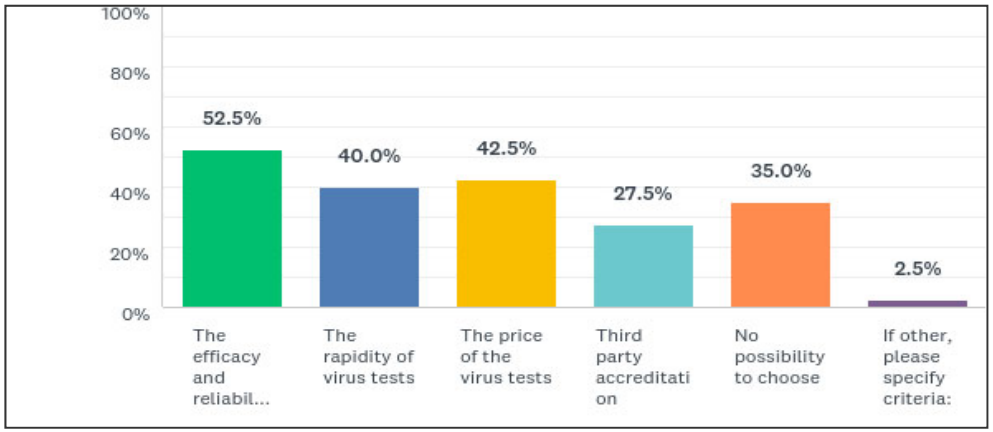
- Only G2 crops and material held in tissue culture must be tested.
- Field testing is exempt since our program is based on visual in the field
- Virus testing is compulsory for all the lots of seed potatoes, before the official certification, with the exception when a field meets all these conditions: The mother seed is 0 % virus, tested by ELISA. The variety is well known. There have been 3 inspections of the field. No virus is observed. This exception was validated by a study carried during 3 years entitled “Viability study of the analysis on leaf to evaluate the virus percentage in seed potatoes for its application in the Basque Country certification.
- Visual inspections are required for all seed. Testing is required for all Nuclear (greenhouse production) and G1 (first year in the field) seed lots. Lab testing PVY nectrotic strains is also required for every lot that has 1.0% or greater mosaic levels at the winter grow out.
- Later generation crops, and certain varieties
- Testing exempt if there are no symptoms seen in the growing crop during official inspections. Leaf samples are taken from growing crops which have symptoms of virus infection. PHT done on one variety only as symptoms not clearly expressed in the field.
- There is an exemption in function of the asked categories of seed potatoes
- All PBTC, PB and Basic material are tested for virus but certified category is not tested in laboratory
- Each year we decide on an exemption date, based on aphid status, for class E and A&B of groups of varieties. If the haulm is killed before this date, virus testing is exempt
- Visual grow out test is performed under foil tent.
- Analyses depends on the registered status for virus resistance, of varieties. Additionally seed potatoes for external markets are analyzed for viruses on request of the exporting firm.
- Routine virus testing is carried out in Pre Basic Crops. In Basic crops it is used to confirm visual findings
- A compulsory virus test is required of for the tissue culture material at the start of the entry of the potato material into the seed certification system. (For the production of nuclear stock class). This virus test must be negative. All remaining virus testing is voluntary unless is part of a foreign country's import requirements.
- PVY testing is done for all crops that are for sale or transfer. Other crops eg early generation not for sale may be done only on voluntary basis
- Certified seed potatoes (class A and class B) are usually not tested

3. Virus testing is done by:



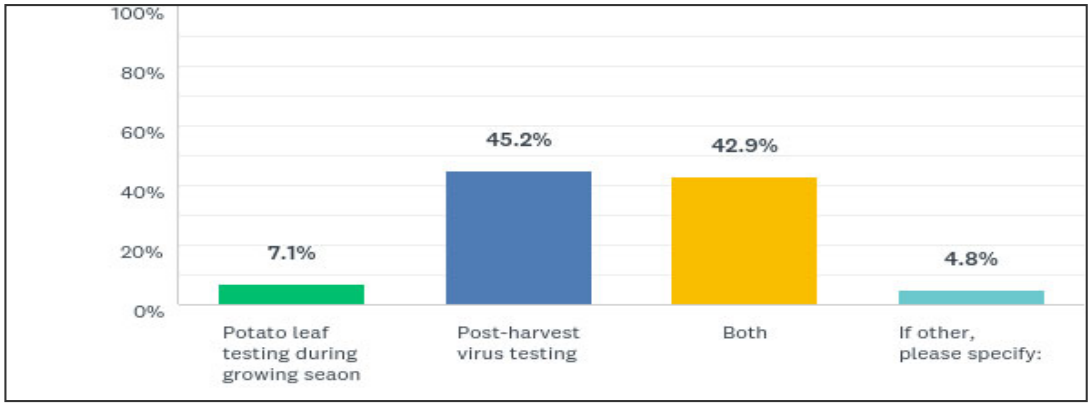
ANSWER CHOICES	RESPONSES	
Your Organization	61.9%	26
Other governmental laboratory	16.7%	7
University or research institute	23.8%	10
Private laboratory	26.2%	11
Laboratory in other country	4.8%	2
If other, please specify:	9.5%	4
Total Respondents: 42		

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



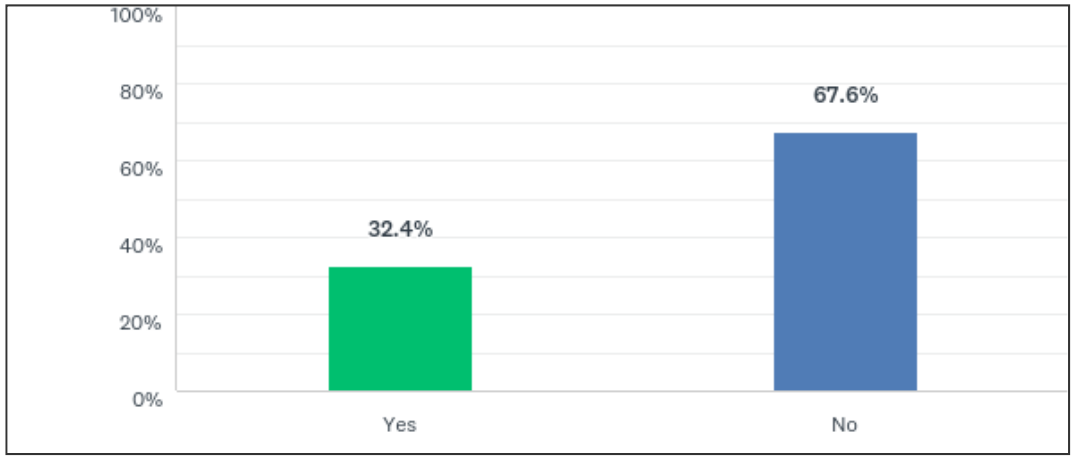
ANSWER CHOICES	RESPONSES	
The efficacy and reliability of virus tests	52.5%	21
The rapidity of virus tests	40.0%	16
The price of the virus tests	42.5%	17
Third party accreditation	27.5%	11
No possibility to choose	35.0%	14
If other, please specify criteria:	2.5%	1
Total Respondents: 40		

5. Type of potato virus testing:



ANSWER CHOICES	RESPONSES	
Potato leaf testing during growing seaon	7.1%	3
Post-harvest virus testing	45.2%	19
Both	42.9%	18
If other, please specify:	4.8%	2
TOTAL		42

6. Does your scheme use UNECE nomenclature?

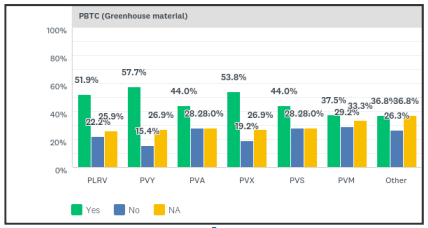


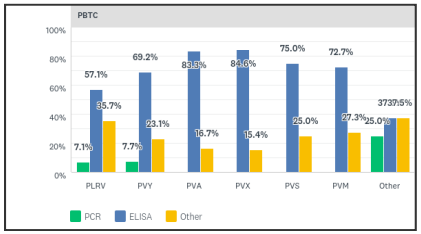
ANSWER CHOICES	RESPONSES	
Yes	32.4%	12
No	67.6%	25
TOTAL		37

6.1 Potato leaf testing during growing season: Seed categories tested, virus tested and the method

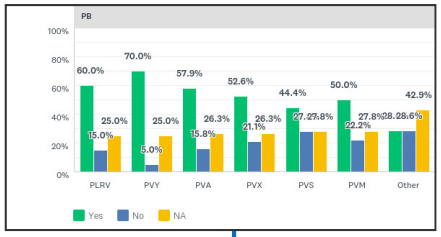
The tables were redesigned according to the seed categories tested: PBTC, PB, Basic and Certified:

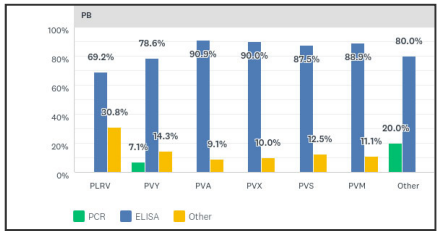
PBTC



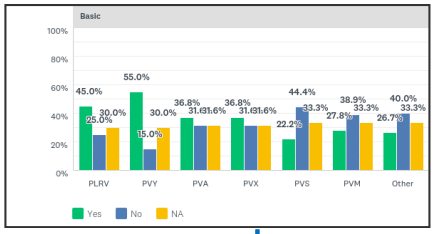


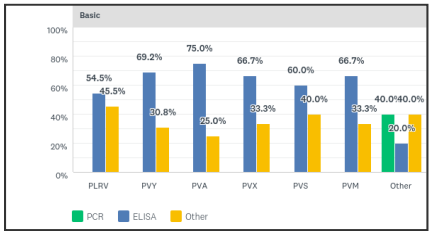
PB



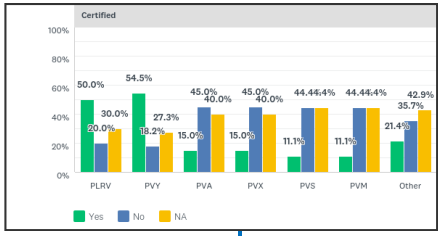


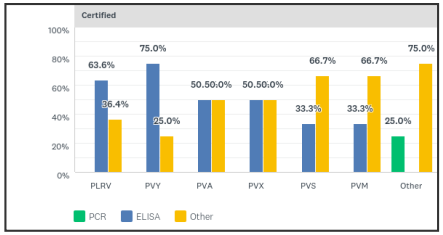
Basic





Certified





The information above is repeated in the next page with a different format that shows the number of respondents for each category.

6.1 Potato leaf testing during growing season: Seed categories tested, virus tested and the method

The tables were redesigned according to the seed categories tested: PBTC, PB, Basic and Certified:

PBTC

PBTC (Greenhouse material)

	YES	NO	NA	TOTAL
PLRV	51.9% 14	22.2% 6	25.9% 7	27
PVY	57.7% 15	15.4% 4	26.9% 7	26
PVA	44.0% 11	28.0% 7	28.0% 7	25
PVX	53.8% 14	19.2% 5	26.9% 7	26
PVS	44.0% 11	28.0% 7	28.0% 7	25
PVM	37.5% 9	29.2% 7	33.3% 8	24
Other	36.8% 7	26.3% 5	36.8% 7	19

PBTC

	PCR	ELISA	OTHER	TOTAL
PLRV	7.1% 1	57.1% 8	35.7% 5	14
PVY	7.7% 1	69.2% 9	23.1% 3	13
PVA	0.0% 0	83.3% 10	16.7% 2	12
PVX	0.0% 0	84.6% 11	15.4% 2	13
PVS	0.0% 0	75.0% 9	25.0% 3	12
PVM	0.0% 0	72.7% 8	27.3% 3	11
Other	25.0% 2	37.5% 3	37.5% 3	8

PB

PB

	YES	NO	NA	TOTAL
PLRV	60.0% 12	15.0% 3	25.0% 5	20
PVY	70.0% 14	5.0% 1	25.0% 5	20
PVA	57.9% 11	15.8% 3	26.3% 5	19
PVX	52.6% 10	21.1% 4	26.3% 5	19
PVS	44.4% 8	27.8% 5	27.8% 5	18
PVM	50.0% 9	22.2% 4	27.8% 5	18
Other	28.6% 4	28.6% 4	42.9% 6	14

PB

	PCR	ELISA	OTHER	TOTAL
PLRV	0.0% 0	69.2% 9	30.8% 4	13
PVY	7.1% 1	78.6% 11	14.3% 2	14
PVA	0.0% 0	90.9% 10	9.1% 1	11
PVX	0.0% 0	90.0% 9	10.0% 1	10
PVS	0.0% 0	87.5% 7	12.5% 1	8
PVM	0.0% 0	88.9% 8	11.1% 1	9
Other	20.0% 1	80.0% 4	0.0% 0	5

Basic

Basic

	YES	NO	NA	TOTAL
PLRV	45.0% 9	25.0% 5	30.0% 6	20
PVY	55.0% 11	15.0% 3	30.0% 6	20
PVA	36.8% 7	31.6% 6	31.6% 6	19
PVX	36.8% 7	31.6% 6	31.6% 6	19
PVS	22.2% 4	44.4% 8	33.3% 6	18
PVM	27.8% 5	38.9% 7	33.3% 6	18
Other	26.7% 4	40.0% 6	33.3% 5	15

Basic

	PCR	ELISA	OTHER	TOTAL
PLRV	0.0% 0	54.5% 6	45.5% 5	11
PVY	0.0% 0	69.2% 9	30.8% 4	13
PVA	0.0% 0	75.0% 6	25.0% 2	8
PVX	0.0% 0	66.7% 6	33.3% 3	9
PVS	0.0% 0	60.0% 3	40.0% 2	5
PVM	0.0% 0	66.7% 4	33.3% 2	6
Other	40.0% 2	20.0% 1	40.0% 2	5

Certified

Certified

	YES	NO	NA	TOTAL
PLRV	50.0% 10	20.0% 4	30.0% 6	20
PVY	54.5% 12	18.2% 4	27.3% 6	22
PVA	15.0% 3	45.0% 9	40.0% 8	20
PVX	15.0% 3	45.0% 9	40.0% 8	20
PVS	11.1% 2	44.4% 8	44.4% 8	18
PVM	11.1% 2	44.4% 8	44.4% 8	18
Other	21.4% 3	35.7% 5	42.9% 6	14

Certified

	PCR	ELISA	OTHER	TOTAL
PLRV	0.0% 0	63.6% 7	36.4% 4	11
PVY	0.0% 0	75.0% 9	25.0% 3	12
PVA	0.0% 0	50.0% 2	50.0% 2	4
PVX	0.0% 0	50.0% 2	50.0% 2	4
PVS	0.0% 0	33.3% 1	66.7% 2	3
PVM	0.0% 0	33.3% 1	66.7% 2	3
Other	25.0% 1	0.0% 0	75.0% 3	4

6.1 Potato leaf testing during growing season: Seed categories tested, virus tested and the method

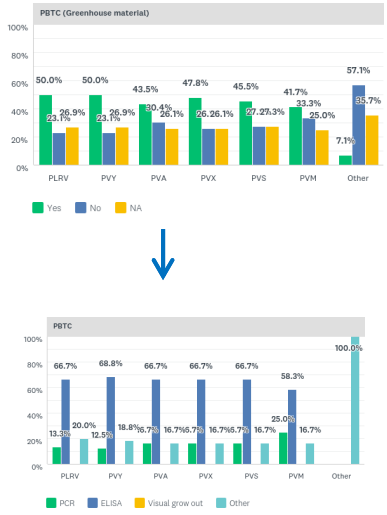
For this question, respondents were asked to specify if «other methods» are used:

- Genebank accessions are all tested for Tomato Spotted Wilt Virus (TSWV) as well and field generations where detected. Growers always have the option to test with ELISA or PCR.
- Only visual check (in doubt using Bioreba Agristrip for confirmation)
- Visual field inspection
- We will use PCR for PVY strain ID, but only on a voluntary basis.
- Only the first generation of prebasic (P2) is tested with leaf testing during growing season. Clones with virus have to be rejected. This testing is conducted by another laboratory (the prebasic centre Overhalla Klonavlssenter AS) than the post-harvest testing for certification purposes, which is done by Fera Science Ltd (UK) for the time being.
- Note: to a small extent, potato leaf testing is carried out during growing season in some very early and early varieties in Schleswig-Holstein. Condition: no virus symptoms are visually observed in the crop. Haulm killing date, no re-growth. Random post-harvest checks are carried out.
- TRV - RT-PCR ELISA - PMTV, TBRV, PVV
- These are only compulsory tests. Other viruses that are tested are PVV and PMTV. During growing season we can test all viruses in all categories, by using ELISA.
- Leaves might in some cases, especially in PBTC and PB be send for analysis by inspectors. For all viruses and all categories PCR is used. Test kit might be used at field inspection.
- For pre-elite need to collect plant samples to do ELISA test, but for the other grade seed only inspect by visual inspection. Note from the Secretariat: The respondent from China replaced the terms used on the top row of the table. "PBTC" was replaced with "Pre-elite", "PB" was replaced with"Elite", "Basic" was replaced with "Qualified I" and "Certified" was replaced with "Qualified II".
- LR,Y,A,X,S,M are all tested using ELISA. Suspect plants only are collected for PBTC and Basic Crops. All PB field stocks have a sample of leaves lifted to test for latent virus infection.
- PALCV = Potato Apical leaf curl virus
- Other is Tomato Spotted Wilt Virus

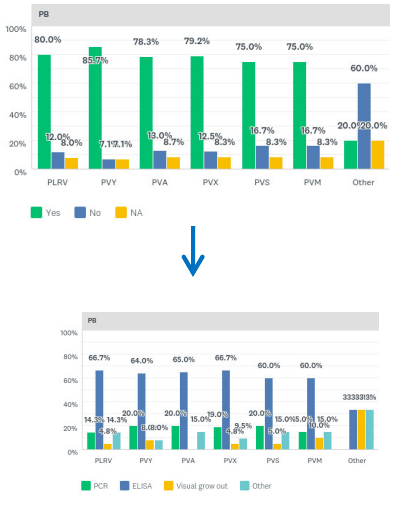
6.2 Post -harvest virus testing, whether by direct tuber testing, sprouts, or leaves in grow out: Seed categories tested, virus tested and the method

The tables were redesigned according to the seed categories tested: PBTC, PB, Basic and Certified:

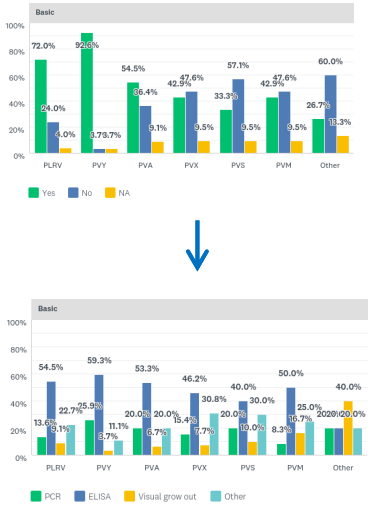
PBTC



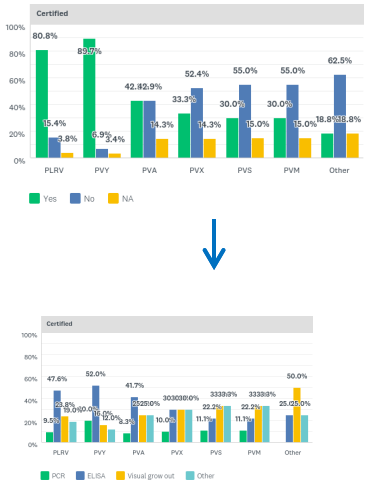
PB



Basic



Certified



The information above is repeated in the next page with a different format that shows the number of respondents for each category.

6.2 Post -harvest virus testing, whether by direct tuber testing, sprouts, or leaves in grow out: Seed categories tested, virus tested and the method

The tables were redesigned according to the seed categories tested: PBTC, PB, Basic and Certified:

PBTC

PBTC (Greenhouse material)

	YES	NO	NA	TOTAL
PLRV	50.0% 13	23.1% 6	26.9% 7	26
PVY	50.0% 13	23.1% 6	26.9% 7	26
PVA	43.5% 10	30.4% 7	26.1% 6	23
PVX	47.8% 11	26.1% 6	26.1% 6	23
PVS	45.5% 10	27.3% 6	27.3% 6	22
PVM	41.7% 10	33.3% 8	25.0% 6	24
Other	7.1% 1	57.1% 8	35.7% 5	14

PBTC

	PCR	ELISA	VISUAL GROW OUT	OTHER	TOTAL
PLRV	13.3% 2	66.7% 10	0.0% 0	20.0% 3	15
PVY	12.5% 2	68.8% 11	0.0% 0	18.8% 3	16
PVA	16.7% 2	66.7% 8	0.0% 0	16.7% 2	12
PVX	16.7% 2	66.7% 8	0.0% 0	16.7% 2	12
PVS	16.7% 2	66.7% 8	0.0% 0	16.7% 2	12
PVM	25.0% 3	58.3% 7	0.0% 0	16.7% 2	12
Other	0.0% 0	0.0% 0	0.0% 0	100.0% 1	1

PB

PB

	YES	NO	NA	TOTAL
PLRV	80.0% 20	12.0% 3	8.0% 2	25
PVY	85.7% 24	7.1% 2	7.1% 2	28
PVA	78.3% 18	13.0% 3	8.7% 2	23
PVX	79.2% 19	12.5% 3	8.3% 2	24
PVS	75.0% 18	16.7% 4	8.3% 2	24
PVM	75.0% 18	16.7% 4	8.3% 2	24
Other	20.0% 3	60.0% 9	20.0% 3	15

PB

	PCR	ELISA	VISUAL GROW OUT	OTHER	TOTAL
PLRV	14.3% 3	66.7% 14	4.8% 1	14.3% 3	21
PVY	20.0% 5	64.0% 16	8.0% 2	8.0% 2	25
PVA	20.0% 4	65.0% 13	0.0% 0	15.0% 3	20
PVX	19.0% 4	66.7% 14	4.8% 1	9.5% 2	21
PVS	20.0% 4	60.0% 12	5.0% 1	15.0% 3	20
PVM	15.0% 3	60.0% 12	10.0% 2	15.0% 3	20
Other	0.0% 0	33.3% 1	33.3% 1	33.3% 1	3

Basic

Basic

	YES	NO	NA	TOTAL
PLRV	72.0% 18	24.0% 6	4.0% 1	25
PVY	92.6% 25	3.7% 1	3.7% 1	27
PVA	54.5% 12	36.4% 8	9.1% 2	22
PVX	42.9% 9	47.6% 10	9.5% 2	21
PVS	33.3% 7	57.1% 12	9.5% 2	21
PVM	42.9% 9	47.6% 10	9.5% 2	21
Other	26.7% 4	60.0% 9	13.3% 2	15

Basic

	PCR	ELISA	VISUAL GROW OUT	OTHER	TOTAL
PLRV	13.6% 3	54.5% 12	9.1% 2	22.7% 5	22
PVY	25.9% 7	59.3% 16	3.7% 1	11.1% 3	27
PVA	20.0% 3	53.3% 8	6.7% 1	20.0% 3	15
PVX	15.4% 2	46.2% 6	7.7% 1	30.8% 4	13
PVS	20.0% 2	40.0% 4	10.0% 1	30.0% 3	10
PVM	8.3% 1	50.0% 6	16.7% 2	25.0% 3	12
Other	20.0% 1	20.0% 1	40.0% 2	20.0% 1	5

Certified

Certified

	YES	NO	NA	TOTAL
PLRV	80.8% 21	15.4% 4	3.8% 1	26
PVY	89.7% 26	6.9% 2	3.4% 1	29
PVA	42.9% 9	42.9% 9	14.3% 3	21
PVX	33.3% 7	52.4% 11	14.3% 3	21
PVS	30.0% 6	55.0% 11	15.0% 3	20
PVM	30.0% 6	55.0% 11	15.0% 3	20
Other	18.8% 3	62.5% 10	18.8% 3	16

Certified

	PCR	ELISA	VISUAL GROW OUT	OTHER	TOTAL
PLRV	9.5% 2	47.6% 10	23.8% 5	19.0% 4	21
PVY	20.0% 5	52.0% 13	16.0% 4	12.0% 3	25
PVA	8.3% 1	41.7% 5	25.0% 3	25.0% 3	12
PVX	10.0% 1	30.0% 3	30.0% 3	30.0% 3	10
PVS	11.1% 1	22.2% 2	33.3% 3	33.3% 3	9
PVM	11.1% 1	22.2% 2	33.3% 3	33.3% 3	9
Other	0.0% 0	25.0% 1	50.0% 2	25.0% 1	4

6.2 Post -harvest virus testing, whether by direct tuber testing, sprouts, or leaves in grow out: Seed categories tested, virus tested and the method

For this question, respondents were asked to specify if «other methods» are used:

- PBTC and the first generation of prebasic (P2) are not tested with post-harvest testing. These two classes are not certified by the authority.
- Note: category certified: depending on susceptibility of the variety, test will be extended to other viruses. most Federal States asses virus by visual assessment in combination with ELISA. In the Federal State Mecklenburg-Vorpommern with high grade areas, all lots are tested for all viruses (ELISA). PCR-Method: For early exports, PCR-tests have been used since 2013 in Lower-Saxony. From 2018 on PCR will be used as the Standard Method in the Federal States Mecklenburg-Vorpommern and Lower-Saxony.
- We use PCR/tuber testing for samples at grower request or if there was a problem with results from field grow out
- PVV - RT-PCR
- PLRV: If suspected
- Note we use a combination of ELISA and Visual inspection for both the Basic and Certified material.
- Testing for PLRV and PVY is compulsory as they have been widely spread. Producer might request for testing for PVA, PVX and PVS. PBTC is in general not tested for virus, as PBTC is produced in protected facilities and environment on micro plants . Micro plants are as well produced in protected facilities and environment as nuclear stock micro plants on material tested and found free from all the above mentioned virus.
- ELISA as a routine method is used for virus detection for tuber leaf after post harvesting, and PCR for tuber test only in the cases that the custom need the urgent result or the ELISA result is suspicious and need to confirm by another method.
- Experimental use of real time PCR method for some specific cases (fast need of results) to assess PVY and PLRV but ELISA continues to be realized in parallel at the current time
- Post harvest virus testing is not routinely carried out.
- The initial virus testing done on tissue culture plantets at the start of entry into the certification system, or if a foreign country import requirement.
- PALCV = Potato Apical leaf curl virus
- Post harvest not routinely used only is issue suspected
- Visual grow out is carried out in post-control plots (100 tubers of each crop) during the next growing season. In case of doubt PCR is carried out on leaves taken from the control plots.

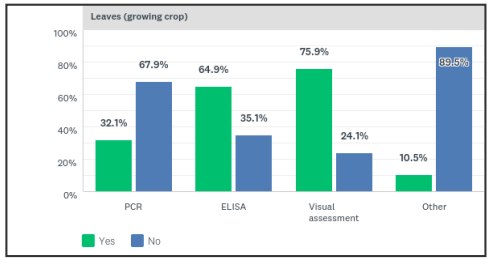
7. Please specify the sample size during the growing season for each diagnostic method (PCR, ELISA or Other Diagnostic Method) and each seed category you use as described above in question 6.1.

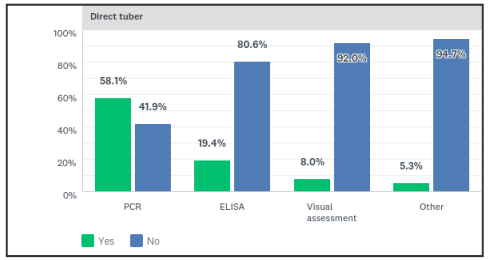
- 100 leaves, 100 sampling points. One leaf selected in top 1/4 of the plant. Group in lots of 10.
- Visual control of the whole surface planted
- ELISA: Basic 100 leaves/≤ ha, Certified 100 leaves/<2 ha, 200 leaves/ > 2 ha (cv. Kennebec, Monalisa, Agria, and Hermes). Rest cultivars 100 leaves/≤ 1 ha, 200 leaves/> 1 ha ≤ 2,5 ha and 300 leaves/> 2,5 ha.
- Basic 400 leaves/faden
- 1% of plant population (greenhouse only)
- ELISA: PBTC 50 leaves min or 1% of plant population, PB 200 min 400 max, Basic 400 lvs, Certified 400 lvs
- ELISA: PBTC NA, PB (first generation; P2) 30-40 leaves per clone, Basic NA, Certified NA
- PB, Basic, Certified: 100 leaves per
- PB 100%, Basic I 10 leaves/family unit, Basic II 200 leaves/acre, Certified 100 leaves/acre
- ELISA 3 compound leaves, up to 6 plants per cop. All grades. Certified NA
- ELISA: all generations 200 leaves
- ELISA: PBTC 1 plantlet, PB +Basic + Certified 110 leaves
- 0,5% of all vitro plants is tested. For ELISA we use 2-4 leaves per reaction.
- PBTC-plantlets, PB-depends on # plants in GH, Basic and Certified varies but typically 400 leaves
- PCR: PBTC+PB+B+Certified NA, ELISA: PBTC+PB+B NA, Certified 500 leaves, Other Diagnostic Method: PBTC+PB+B+Certified NA
- PBTC - 200 leaves
- Suspected virus samples
- Grow out test: Tuber selected 0.3% of the lot from pre basic material (breeders seed). Visual monitoring for all virus symptoms
- Suspect samples – one leaf per plant. Routine samples PB stocks only) – 50 to 100 leaflets per stock. All samples are tested by ELISA
- 6 test tube plantlets
- 200 leaves
- PCR: PBTC 100% of plants, PB 200 leaves, Basic 200 leaves, Certified 100 leaves, ELISA: PBTC 100% of plants, PB 200 leaves, Basic 200 leaves, Certified 100 leaves
- PCR: PBTC 200 LEAVES/ELISA:PBTC+PB+B 200 LEAVES Y CERT 100 LEAVES

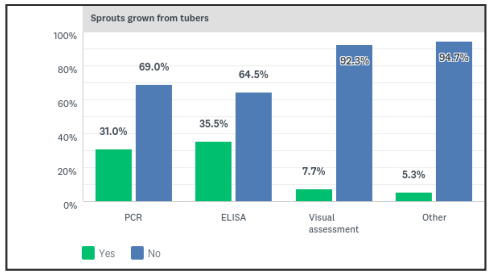
8. Please specify the tuber sample size for each diagnostic method (PCR, ELISA, Visual Grow Out or Other Diagnostic Method) and each seed category you use as described above in question 6.2 (whether by direct tuber testing, sprout testing or grow out testing).

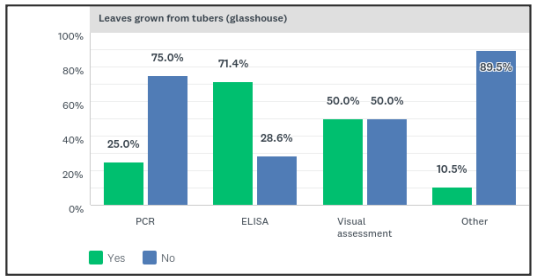
- PCR: PBTC 1%, ELISA: G0 2 tubers per 100 plants ELISA: Basic - G1, G2 400 tubers per 2.5 hectares, G3, 400 tubers per 5 hectares, G4, G5, G6, G7 ELISA: Certified - G8, 200 tubers per 5 hectares
- PCR: PBTC 96 tubers, PB 200 tubers, Basic 200 or 296 tubers (depends on the sensibility of the cultivar and field size), Certified 96, 200 or 296 tubers (depends on the sensibility of the cultivar and field size)
- ELISA: PBTC + PB + Basic 100 tubers/≤1ha, Certified 100 tubers/≤1ha, 200 tubers/> 1 ha ≤ 2,5 ha and 300 tubers/ > 2,5 ha.
- 100 tubers
- ELISA: PB 110 tubers, PCR : Basic+Certified 110 tubers
- VISUAL GROW OUT and ELISA: PB + BASIC up to 4 ha 220 tubers, up to 10 ha 330 tubers, over 10 ha 440 tubers, Certified category: A up to 5 ha 110 tubers, up to 10 ha 220 tubers, over 10 ha 330 tubers, category B: up to 10 ha 110 tubers, over 10 ha 220 tubers.
- ELISA: PBTC + PB + Basic 200 tubers, Certified 100 tubers
- ELISA: PBTC + PB + Basic 120 tubers.
- Basic 400 tubers
- ELISA: PBTC + PB + Basic 250 tubers, Certified 150 tubers
- 400 tubers (PCR and ELISA)
- ELISA: Basic and Certified 400 lvs, Visual GO: Basic and Certified 600 tubers
- ELISA: PBTC NA, PB (P3-P4) + Basic + Certified 100 tubers.
- ELISA and visual test (grow out): 100 tubers per 3 ha (all categories) are tested
- 400 tubers, all lots
- PCR 150 tubers. FG5 and below. Certified NA.
- PCR: PB : NA; B: 110 tub (less or equal 5ha), 220 tub (more 5 ha); Cert.: 110 tub (less or equal 10 ha), 220 tub (more 10 ha). ELISA: PB: 550 tub; B: 220 tub (less or equal 5 ha), 440 tub (more 5 ha); Cert.: 110 tub (less or equal 10 ha), 220 tub (more 10 ha) (NC = not compulsory)
- ELISA: PBTC 1 plantlet, PB +Basic + Certified 110 tubers
- PCR: PB, S,SE 300/5 Ha, E 200/5 Ha, A 100/10 ha
- ELISA: PBTC, PB, Basic 100 tubers, Certified NA. PCR: PBTC, PB, Basic 100 tubers, Certified NA
- PCR: PB 200, Basic 200, Certified 200
- ELISA: each category 100 leaves from 200 tubers Visual grow out 200 tubers
- We don't test tubers-only leaf material or plantlets
- PCR: PBTC+PB+B+Certified NA, ELISA: PBTC+PB+B NA Certified 100 tubers, Visual Grow Out: PBTC+PB+B+Certified NA, Other Diagnostic Method: PBTC+PB+B+Certified NA
- ELISA, CERTIFIED 100 TUBERS
- PCR: PB: 100+100 tubers for each ha, of one single plot of each variety, Basic and C: one sample of 100 tuber, for each 7 ha of one single plot of each variety. If plot less than 7 ha: at least one sample of 100 tubers.
- PBTC, PB, B = 220 tubers; (more than 1 ha = 330 Tubers) ; C = 110 tubers
- ELISA: from 110 up to 350 tubers (depending on the size of the lot); all categories
- PBTC : 1 tuber per 20 plants of the same lot; PB : 200 to 400 tubers according the crop area; Basic : 200 to 400 tubers according the crop area and the grade; Certified : 100 to 200 tubers according the crop area
- Grow out test: Pre basic Tuber selected 0.3% of the lot from pre basic material (breeders seed)
- ELISA: CERTIFIED 120 TUBERS, BASIC 120 TUBERS
- 200 tubers
- PB and basic class S: crop ≤ 100a 125 tubers / 100a < crop ≤ 200a 2 x 125 tubers /200a < crop 3 x·125 tubers; basic classes SE and E: crop ≤ 150a 125 tubers / 150a < crop ≤ 400a 2·x 125 tubers / 400a < crop 3· 125 tubers
- PCR: PBTC 100% of plants, PB 200 tubers, Basic 100 tubers, Certified 100 tubers; ELISA: PBTC 100% of plants, PB 200 tubers, Basic 100 tubers, Certified 100 tubers; Visual Grow Out: PBTC 200 tubers, PB 200 tubers, Basic 200 tubers, Certified 200 tubers
- ELISA: PB+B 200 TUBERS CERT 100 TUBERS

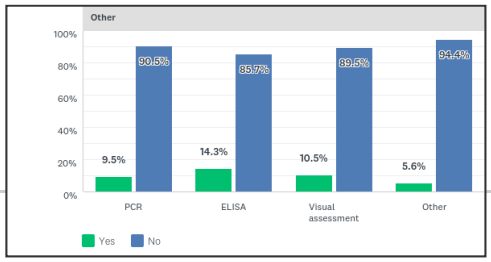
9. Please specify, using YES or NO, the official test methods used for assessing virus infection, whether by leaf testing during growing season, direct tuber testing, sprouts, or leaves in grow out.





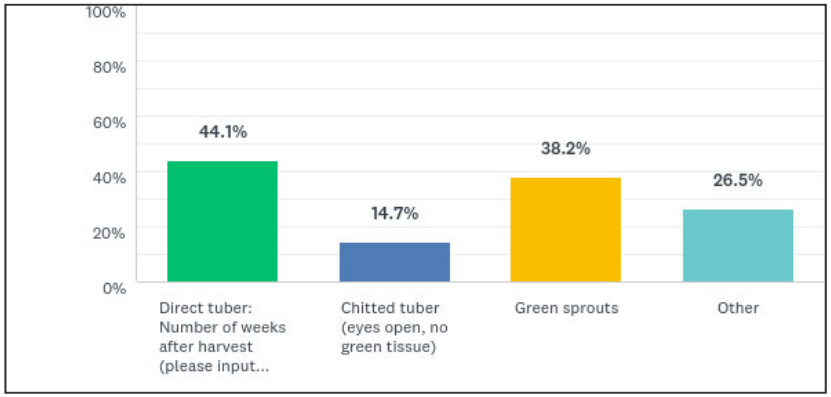






Leaves (growing crop)	YES	NO	TOTAL
PCR	32.1% 9	67.9% 19	28
ELISA	64.9% 24	35.1% 13	37
Visual assessment	75.9% 22	24.1% 7	29
Other	10.5% 2	89.5% 17	19
Direct tuber	**YES**	**NO**	**TOTAL**
PCR	58.1% 18	41.9% 13	31
ELISA	19.4% 6	80.6% 25	31
Visual assessment	8.0% 2	92.0% 23	25
Other	5.3% 1	94.7% 18	19
Sprouts grown from tubers	**YES**	**NO**	**TOTAL**
PCR	31.0% 9	69.0% 20	29
ELISA	35.5% 11	64.5% 20	31
Visual assessment	7.7% 2	92.3% 24	26
Other	5.3% 1	94.7% 18	19
Leaves grown from tubers (glasshouse)	**YES**	**NO**	**TOTAL**
PCR	25.0% 7	75.0% 21	28
ELISA	71.4% 25	28.6% 10	35
Visual assessment	50.0% 13	50.0% 13	26
Other	10.5% 2	89.5% 17	19
Other	**YES**	**NO**	**TOTAL**
PCR	9.5% 2	90.5% 19	21
ELISA	14.3% 3	85.7% 18	21
Visual assessment	10.5% 2	89.5% 17	19
Other	5.6% 1	94.4% 17	18

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



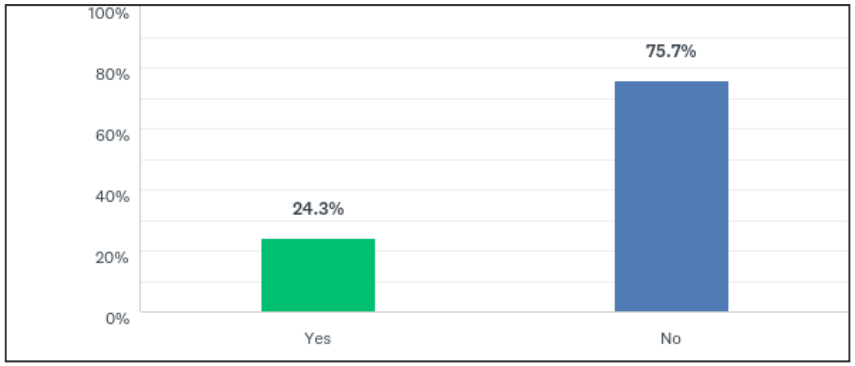
ANSWER CHOICES	RESPONSES	
Direct tuber: Number of weeks after harvest (please input number into the text box below)	44.1%	15
Chitted tuber (eyes open, no green tissue)	14.7%	5
Green sprouts	38.2%	13
Other	26.5%	9
Total Respondents: 34		

Respondents that chose «Direct Tuber» were required to input the number of weeks after harvest, and respondents that chose «Other» were asked to specify:

- Direct tuber for PCR testing - 2 weeks, ELISA - Green sprouts - after 28 days if sprouted or longer, sprouts must be at least 3-5mm long
- 8 to 12 weeks after harvest of single tubers (sampling 1 tuber per plant)
- 9
- Bits of tubers are planted in peat in glasshouse and grown for 6-8 weeks. This is done during October-December.
- Directly after harvest
- not regulated, usually at least 4 weeks
- Post desiccation and pre harvest
- 0 - 3 weeks
- Leaves in grown out.
- If possible immediately after harvest, depending on weather / organisational issues etc.
- We only test if tuber is grown out and leaves are removed for testing
- Immediately after the harvest
- Tubers are analyzed between 0 and 2-6 month after harvest. Sampling is primarily done after withering but before harvest. Sampling at storage is possible
- We mainly according to the customer request and complete testing time, determine to inspect the status of tuber. If there are no demands that we don't usually direct detection tubers.
- Direct tuber: Leaves grown from tubers (glasshouse) Experimental use of qPCR: No impact of physiological stage of potato tuber.
- Tubers are tested directly after harvest
- 8 TO 12 WEEKS

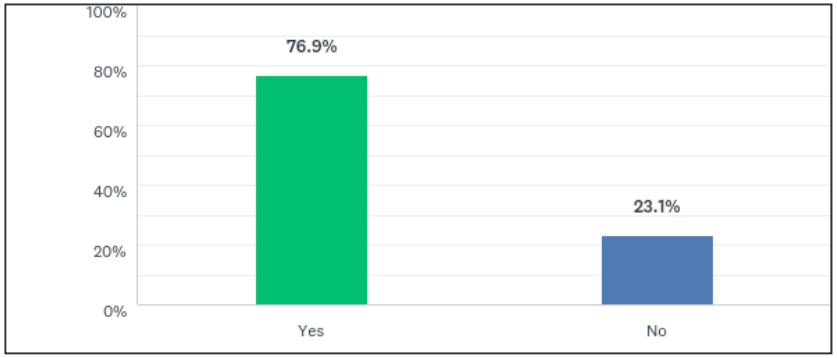
11. If ELISA is used in the laboratory, how was it developed? Please provide answers to questions 11.1, 11.2 and 11.3 below.

11.1 In-house developed method.



ANSWER CHOICES	RESPONSES	
Yes	24.3%	9
No	75.7%	28
TOTAL		37

11. If ELISA is used in the laboratory, how was it developed? Please provide answers to questions 11.1, 11.2 and 11.3 below.
11.2 Commercial kit method (non-exhaustive list of suppliers)

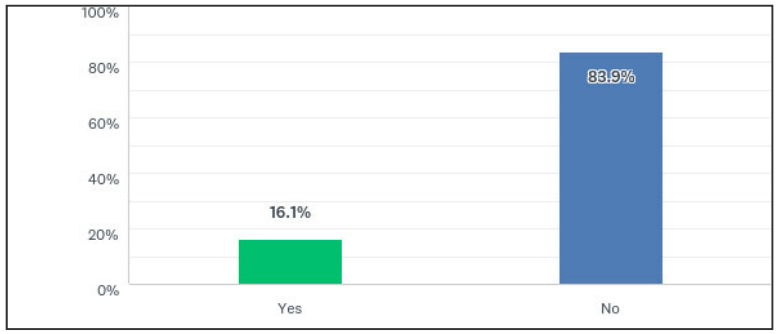


ANSWER CHOICES	RESPONSES	
Yes	76.9%	30
No	23.1%	9
TOTAL		39

Respondents that selected «yes» were required to specify the supplier:

- Agdia (9)
- Adgen (1)
- Bioreba (16)
- Biomedica (1)
- Loewe Biochemica GmbH, DSMZ Germany (2)
- LTD "Hanbit“ (1)
- Neogen (3)
- Plant Research International (Prime Diagnostics) (1)
- VNIIKH (1)

11. If ELISA is used in the laboratory, how was it developed? Please provide answers to questions 11.1, 11.2 and 11.3 below.
11.3 Other.

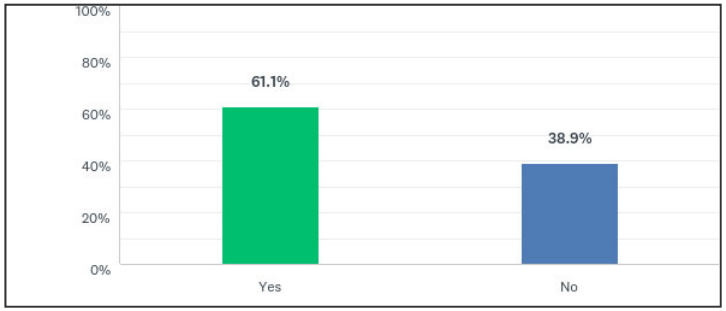


ANSWER CHOICES	RESPONSES	
Yes	16.1%	5
No	83.9%	26
TOTAL		31

Respondents that selected «yes» were required to specify:

- SASA Scotland and MPI for PVY strains (when requested, not an official requirement).
- We use monoclonals developed in house
- Plant Research International
- NAK Testing Protocol 1995

12. Are the samples pooled for ELISA testing?



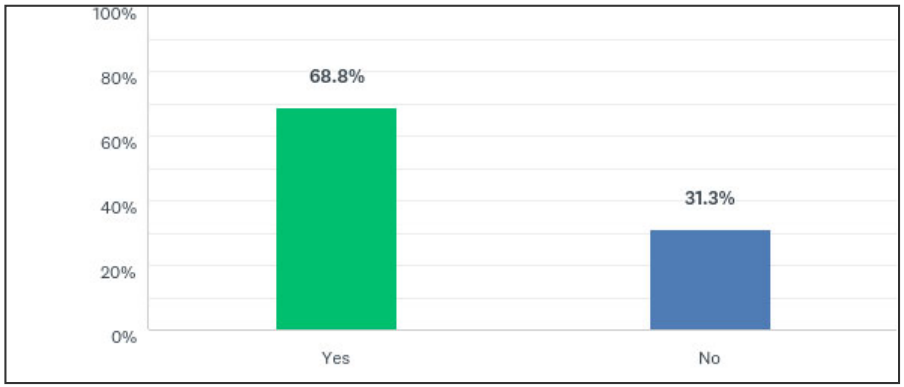
ANSWER CHOICES	RESPONSES	
Yes	61.1%	22
No	38.9%	14
TOTAL		36

Respondents that selected «yes» were required to specify the total number of subsamples.

- 5
- 10*10 leaves
- 24 (pooled 4 samples together)
- 100
- 5
- Leaves are pooled by groups of 5.
- 10 leaves per well
- 25 (with 4 plants/tubers in each subsample)
- Standard is NO, only under certain conditions: 4 leaf test: 25 x 4
- 10
- 110
- 20 (20 x 5 = 100)
- Depends on the total number of plants tested, we pool 2-4 leaves per reaction
- Ten leaves are pooled to create one sample
- In case of testing leaves 100 subsamples for certified crops. In case of testing tubers 50 subsamples for certified lots or without pooling (100 tubers tested).
- 45
- 4 samples in 1 reaction for Elisa
- For each sample of 200 to 400 tubers, subsamples of 5 pooled leaves (grown from tubers in glasshouse) are tested. So between 40 to 80 subsamples per sample (1 sample = 1 crop).
- 3
- They are composited, not pooled.
- 20
- 2-4 (100x2; 50x2; 25x4; 50x4)
- 5

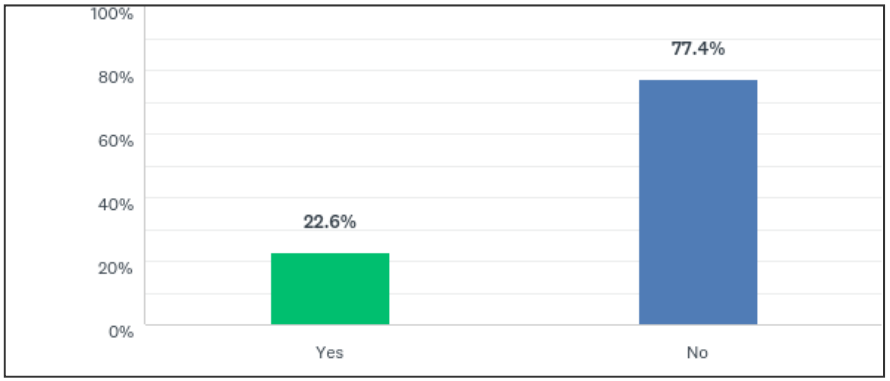
13. If PCR is used in the laboratory, how was it developed? Please provide answers to questions 13.1, 13.2 and 13.3 below.

13.1 In-house developed method.



ANSWER CHOICES	RESPONSES	
Yes	68.8%	22
No	31.3%	10
TOTAL		32

13. If PCR is used in the laboratory, how was it developed? Please provide answers to questions 13.1, 13.2 and 13.3 below.
13.2 Commercial kit method (non-exhaustive list of suppliers)

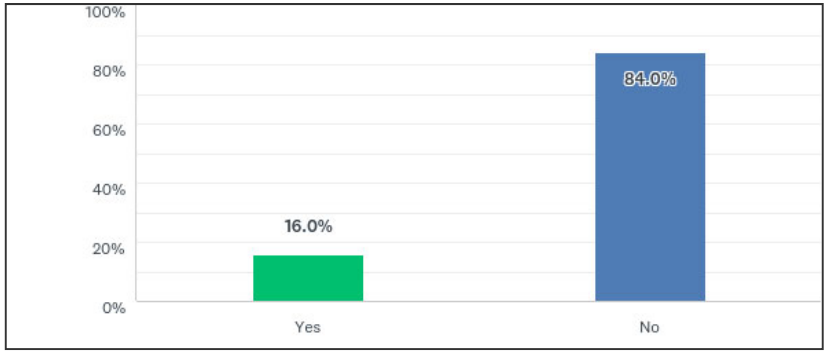


ANSWER CHOICES	RESPONSES	
Yes	22.6%	7
No	77.4%	24
TOTAL		31

Respondents that selected «yes» were required to specify supplier:

- Thermo Scientific verso 1-step RT-PCR
- DNA-Technology; LTD "Agrodiagnostika"

13. If PCR is used in the laboratory, how was it developed? Please provide answers to questions 13.1, 13.2 and 13.3 below.
13.3 Other.

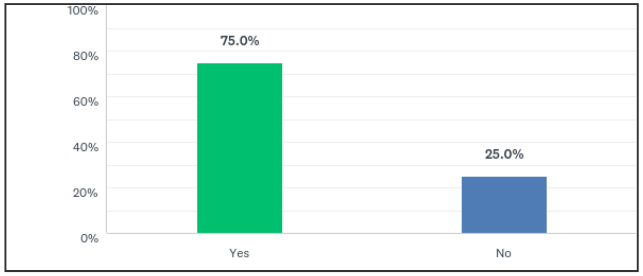


ANSWER CHOICES	RESPONSES	
Yes	16.0%	4
No	84.0%	21
TOTAL		25

Respondents that selected «yes» were required to specify:

- PCR testing done at NDSU Diagnostic Lab, (in house developed) not at ND Seed Lab.
- Real time PCR methodologi
- The method is developed by Fera (UK)
- Obtained primers etc from published papers and then optimized the protocols to our needs.

14. Are the tubers/leaves pooled/bulked for PCR testing?



ANSWER CHOICES	RESPONSES	
Yes	75.0%	24
No	25.0%	8
TOTAL		32

If the answer is yes, respondents were required to input the total number of subsamples for both categories.

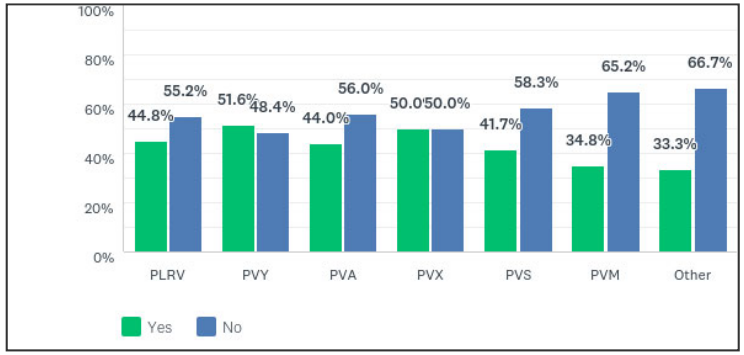
Leaf samples

- 5
- 5 leaf composites
- 25
- 10
- 0
- 10 leaves per sample
- 50
- 10
- 3-5
- They are composited, not pooled.
- 20
- 25x4; 25x8
- 10 TO 30

Tuber samples

- 5
- 8 bulks (subsample size 12, 25, and 37 tubers, according to the total sample size 100, 200, and 300 tubers)
- 10
- 25 subsamples with 4 tubers in each (as in the growing out ELISA testing)
- at present only for early export: 10 x 10 / from 2018: 8 x 25
- 10
- 10 tubers per bulk
- 110
- 100 tubers/sprouts in 10 groups
- 10 (10 x 10 =100)
- 4
- 50
- 10
- 4
- bulks of 20 tubers
- 4 OR 10
- They are composited, not pooled.
- 20
- 6-20 tubers
- 25x4; 25x8
- 10 TO 30

15. Are the PCR primer sequences publicly available for use? Please specify by virus in table.



	YES	NO	TOTAL RESPONDENTS
PLRV	44.8% 13	55.2% 16	29
PVY	51.6% 16	48.4% 15	31
PVA	44.0% 11	56.0% 14	25
PVX	50.0% 13	50.0% 13	26
PVS	41.7% 10	58.3% 14	24
PVM	34.8% 8	65.2% 15	23
Other	33.3% 5	66.7% 10	15

Respondents were also asked to provide references for the primer sequences:

•Boonham N, Laurenson L, Weekes R et al (2009) Direct detection of plant viruses in potato tubers using real-time PCR.
•D.P. Maxwell. Development of highly sensitive multiplex reverse transcription polymerase chain reaction (m-RT-PCR) method for detection of three potato viruses in a single reaction and nested PCR. Arab. J. Biotech. Vol. 5, No. (2) July (2002):275-286
•Simultaneous Detection of Potato Viruses, PLRV, PVA, PVX and PVY From Dormant Potato Tubers by TaqMan Real-Time RT-PCR BO Agindotan et al. J Virol Methods 142 (1-2), 1-9. 2007 Feb 05. more
•Other - PVV and TRV. Reference - Lacomme C, Holmes R, Evans F (2015). Molecular and serological methods for the diagnosis of viruses in potato tubers. In. Plant Pathology: techniques and Protocols 2nd Edition. Methods in Molecular Biology, vol.1302. Ed Christophe Lacomme. Springer.
•Direct tuber testing. Fera Project No. VQ22 1060.
•Humphris SN, Cahill G, Elphinstone JG, Kelly R, Parkinson NM, Pritchard L, Toth IK, Saddler GS. 2015. Detection of the bacterial potato pathogens Pectobacterium and Dickeya spp. using conventional and real-time PCR. Methods in Molecular Biology 1302:1-16.
•Development and application of a universal and simplified multiplex RT-PCR assay to detect five potato viruses (NY/T 2678-2015 Detection of the six potato viruses-RT-PCR method ）
•Kogovsek et al, 2008, Bright et al, 2006
•Souze-Dias et al. (1999)
•PVY an PLRV: Boonham et al. (2009) ; PVS and PVX: Mortimer-Jones et al. (2009); PVA: Lacomme (2015)

16. How are the results statistically interpreted for use in certification (e.g., ISTA seedcalc)?

- ISTA Seedcalc (12 answers)
- Direct percentage (6 answers)

Others:

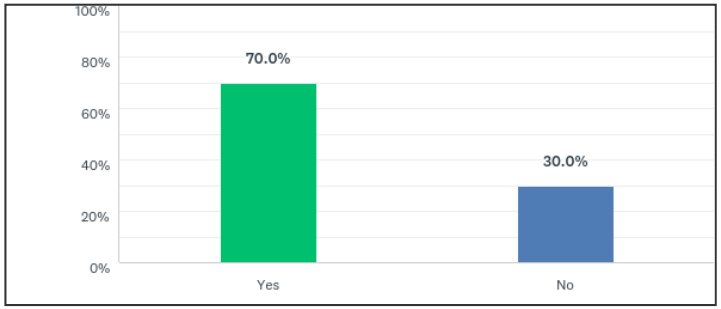
- Statistical tables obtained internationally
- Gibbs and Gowers (1960) formula.
- UNECE S-1
- Serological results compared against visual readings to determine classification based on respective tolerances. Exception is for viruses that do not express visible symptoms. Serological test results determine acceptance.
- ISTA seedcalc8 Percentage is based on total of number of composite testes of 10 leaves/tubers. Formula is: %virus=+(1-(1-A4/B4)^(1/10))*100
- Flemish standards for the seed potatoes certification
- ELISA results are %-results, ISTA SeedCalc 8 is used for PCR
- PCR = ISTA, ELISA = no statistical interpretation (1:1)
- They are calculated based on published research that allow for sampling of 10 leaves per sample. J. Phytopathology 144. 459-463 (1996). Clarke, R.G et al. Plant Disease 64: 43-45.
- FOLLOW NATIONAL REGULATION HARMONIZED WITH THE RELEVANT EU AQCUIS
- DANAK accreditation is used.
- Statistical tables similar to ISTA seedcalc
- The tests must be negative. There is no statistics that is done. If the test is not negative, the material is discarded.
- ГОСТ Р 53136-2008 ГОСТ 33996-2016 (from 2018-01-01)

17. How does the authority use the lab result to determine the classification of the crop? I. Please supply the classification table, including how the results determine the class of the crop:

UNECE Seed Potato Standard http://www.unece.org/trade/agr/standard/potatoes/pot_e.html

List of National Certification Schemes for Seed Potatoes
http://www.unece.org/trade/agr/standard/potatoes/pot_e.html

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

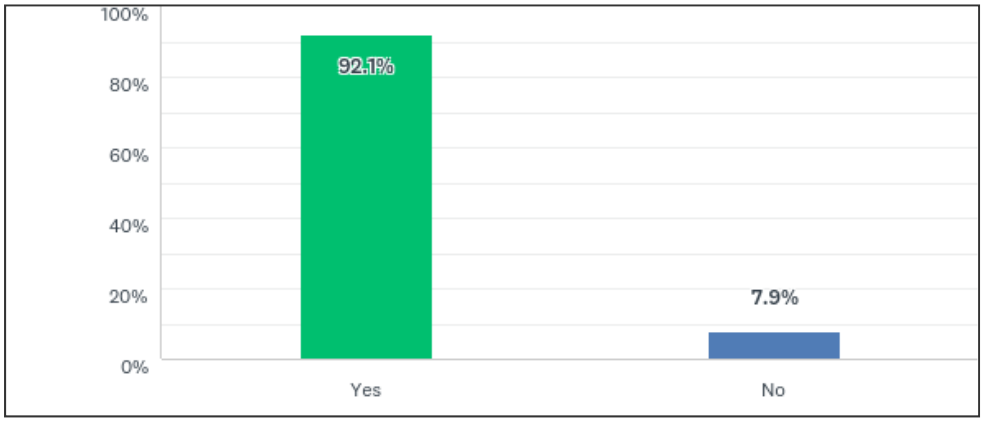


ANSWER CHOICES	RESPONSES	
Yes	70.0%	28
No	30.0%	12
TOTAL		40

If the answer is yes, respondents were required to specify the accreditation/approval body:

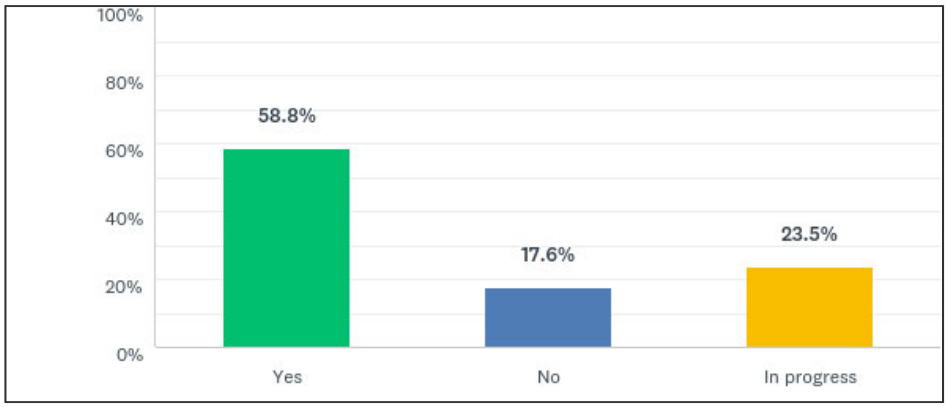
- Independent Certification Council for Seed Potatoes, Department of Agriculture, Forestry and Fisheries, University of Stellenbosch
- Approved by the NZ Seed Potato Certification Authority. Also laboratory accredited (rather than specific tests) by MPI.
- AENOR
- Estonian Accreditation Body
- Polskie Centrum Akredytacji (Polish Accreditation Body)
- USDA
- USDA-APHIS
- United Kingdom Accreditation Service (Fera holds accreditation to UKAS ISO17025, and management of their operations is certified by ISO9001)
- DAkkS (Deutsche Akkreditierungsstelle)
- UKAS - 17025
- Czech Institute for Accreditation
- The laboratory also needs an quality assurance system
- SWEDAC
- National Accreditation Authority
- We are certified by DATCP.
- DANAK
- Ministry of agriculture, Forestry and food
- AKKREDITIERUNG AUSTRIA
- Government: Chinese Bureau of Technical Supervision(CSBTS), Chinese Ministry of Agriculture(CMoA)
- Accreditation ISO 17025 by the National accreditation body (COFRAC) + Approval by Ministry of Agriculture
- Department of Biotechnology, New Delhi
- SGS
- ISO17025 and ICC
- NATA
- System of voluntary certification “Russian Agricultural Center”

Does the laboratory have an internal Quality Control System?



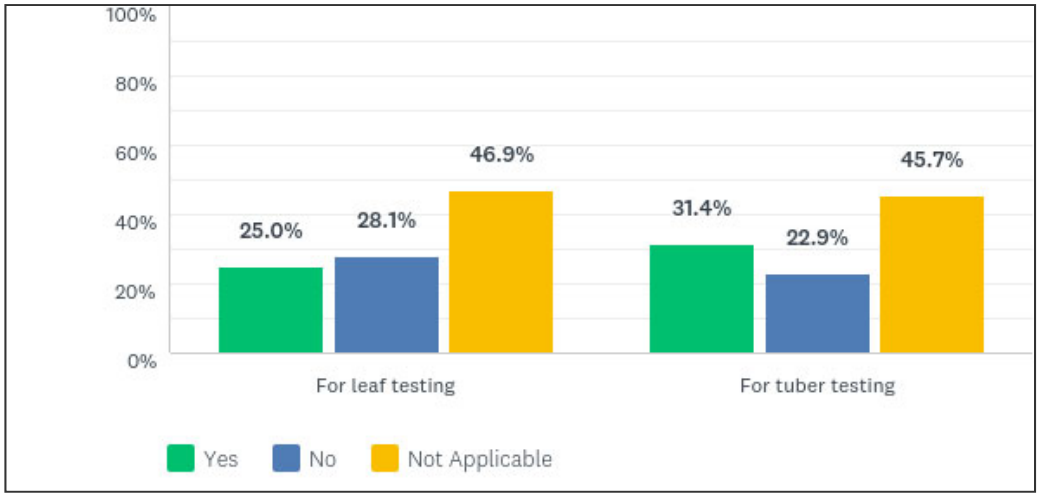
ANSWER CHOICES	RESPONSES	
Yes	92.1%	35
No	7.9%	3
TOTAL		38

Has the laboratory validated their PCR virus testing method?



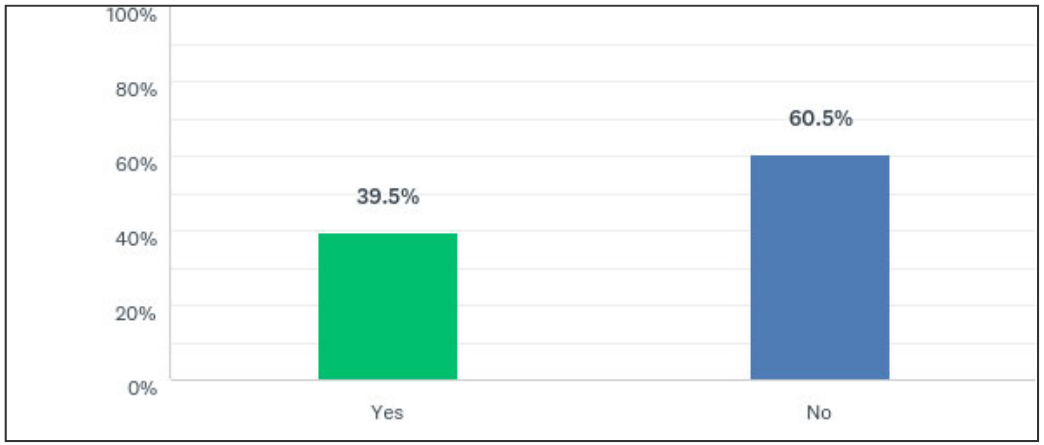
ANSWER CHOICES	RESPONSES	
Yes	58.8%	20
No	17.6%	6
In progress	23.5%	8
TOTAL		34

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



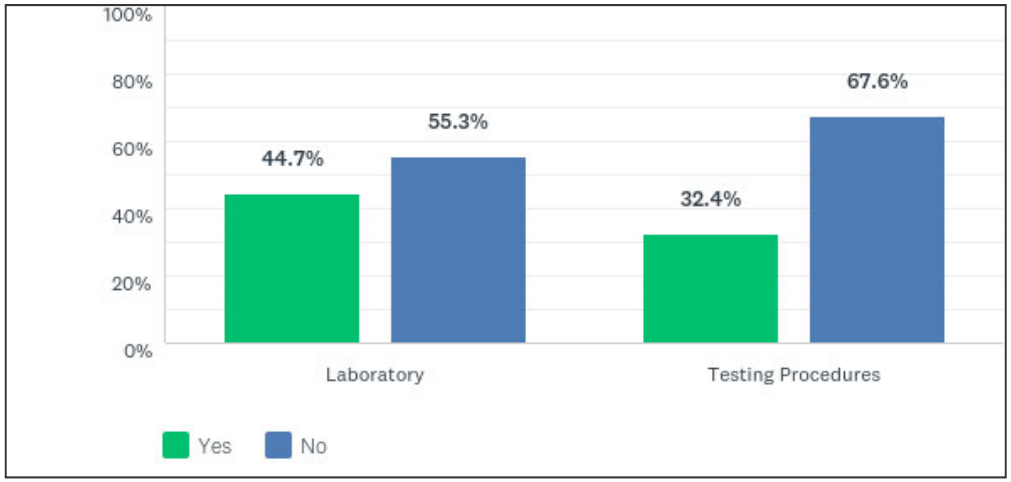
	YES	NO	NOT APPLICABLE	TOTAL
For leaf testing	25.0% 8	28.1% 9	46.9% 15	32
For tuber testing	31.4% 11	22.9% 8	45.7% 16	35

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



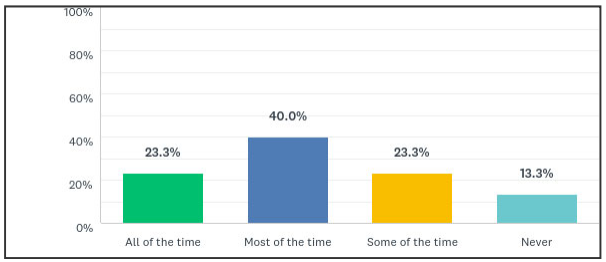
ANSWER CHOICES	RESPONSES	
Yes	39.5%	15
No	60.5%	23
TOTAL		38

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



	YES	NO	TOTAL	WEIGHTED AVERAGE
Laboratory	44.7% 17	55.3% 21	38	1.55
Testing Procedures	32.4% 12	67.6% 25	37	1.68

22. In your experience, does ELISA on sprouted tubers and direct tuber test by PCR give equivalent results?

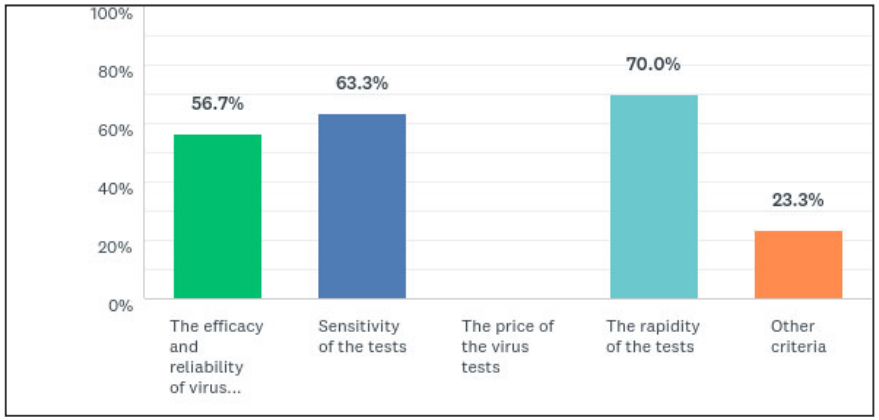


ANSWER CHOICES	RESPONSES	
All of the time	23.3%	7
Most of the time	40.0%	12
Some of the time	23.3%	7
Never	13.3%	4
TOTAL		30

Respondents were asked to explain their answers:

- Sensitivity of PCR allows detection of more recent infections
- Equivalent results in 99.5% of the tested lots (equal category)
- We have not compared ELISA from sprouted tubers and direct tuber test by PCR but we have compared direct tuber testing by Real-Time RT-PCR and ELISA from grown plants and the results were comparable most of the time
- Limited experience. Direct tuber testing with protocol for sprout testing has consistently shown false negatives.
- Norwegian Food Safety Authority has not much experience, but 'most of the time' is the experience of Fera. In some cases results have differed, generally when Fera has seen differences in the specificity of the assays being used. This may either be due to antisera specificity or sensitivity of the methods. Antisera may in some cases have a broader range of detection than the PCR primers.
- Tests have shown equivalent results for >98 % of the lots. As PCR is more sensitive, slightly more positive results with PCR were observed.
- Internal experiments have demonstrated that PCR testing of tuber tissue is 86-89% as sensitive as ELISA for sprouted tubers
- A 5-year survey of Estima PHT by both Real Time RT-PCR and ELISA growing on methods gave comparable outcome
- Earlier there were some problems, for example cause by chemicals chosen
- Direct tuber testing is more reliable than testing on sprouted tubers. Before we did PCR, we used ELISA, but never on sprouted tubers (we used leaves in grow out)
- Some double sampling and analysis has shown to give equivalent results
- Most of the time , does ELISA on sprouted tubers and Number of weeks after harvest of tuber test by PCR give equivalent results。 But, i do not know how many weeks we need.
- COMPARISON IN PROGRESS. WE HAVE EQUIVALENT RESULTS ON REFERENCE INFECTED AND HEALTY SAMPLES
- we have had variable results on direct tuber testing using PCR
- The same positive results
- ELISA TEST FOR PLRV IN TUBER OR SPROUTS IS NOT GOOD METHOD

23. If you use PCR, please explain what the advantages are to your certification system:



ANSWER CHOICES	RESPONSES	
The efficacy and reliability of virus tests	56.7%	17
Sensitivity of the tests	63.3%	19
The price of the virus tests	0.0%	0
The rapidity of the tests	70.0%	21
Other criteria	23.3%	7
Total Respondents: 30		

ADDITIONAL COMMENTS MADE BY RESPONDENTS

- Our challenge is reliably detecting primary infection by the direct tuber test (PCR). We are working on the method but would welcome other countries thoughts on tissue sampling, storage time etc.

- We choose different methods for different kinds of sample. For example leaf test by ELISA, direct tuber test by PCR. Plantlet in-vitro test by both of them. The customers should be told the test method used.