LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture, de la Viticulture
et du Développement rural

Administration des services techniques
de l'agriculture

EPPO Workshop for Heads of Plant Pest Diagnostic Laboratories

Oeiras (PT), 2023-04-19/20

A review of proficiency tests in plant pathology in the Luxembourgish NRL by the example of potato viruses

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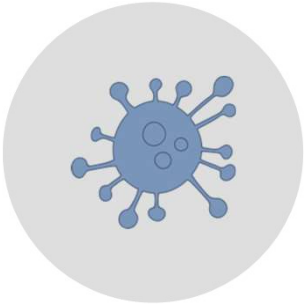
Content

Luxembourgish NRL

ASTA – department of phytopathology



PT - potato viruses

A short overview

Applied method

Participation in PTs

Key facts

Quality management

PTs as an indicator

Who we are

ASTA – department of phytopathology

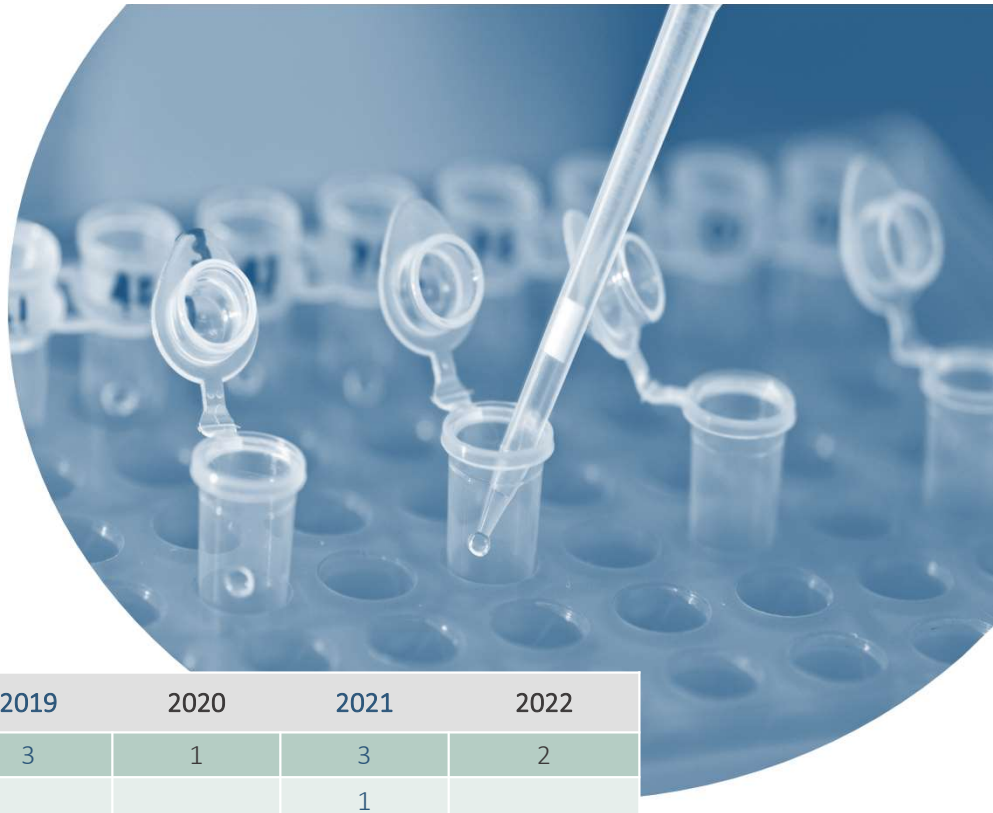
The Administration of technical agricultural services (ASTA - *Administration des services techniques de l'agriculture*) operates under the authority of the Luxembourgish Ministry of Agriculture.

ASTA's tasks are amongst others the control of contents and quality of agricultural products in order to advise or consult the sector. Its aim is the promotion of a conscientious and sustainable agriculture in accordance with the legislative and regulatory stipulations.

Our missions

- ✓ Phytodiagnostics
  - Quarantine Pests
  - RNQPs (Regulated Non-Quarantine Pests)
  - Others
- ✓ Scientific and analytical assistance for the official seed certification
- ✓ Consultation and advice
- ✓ National Reference Laboratory for plant health

Overview of participation in various PTs



Participation in PTs since 2017

PTs in bacteriology, fungi, virology, nematology and for insects

PTs from EURL or other organizers

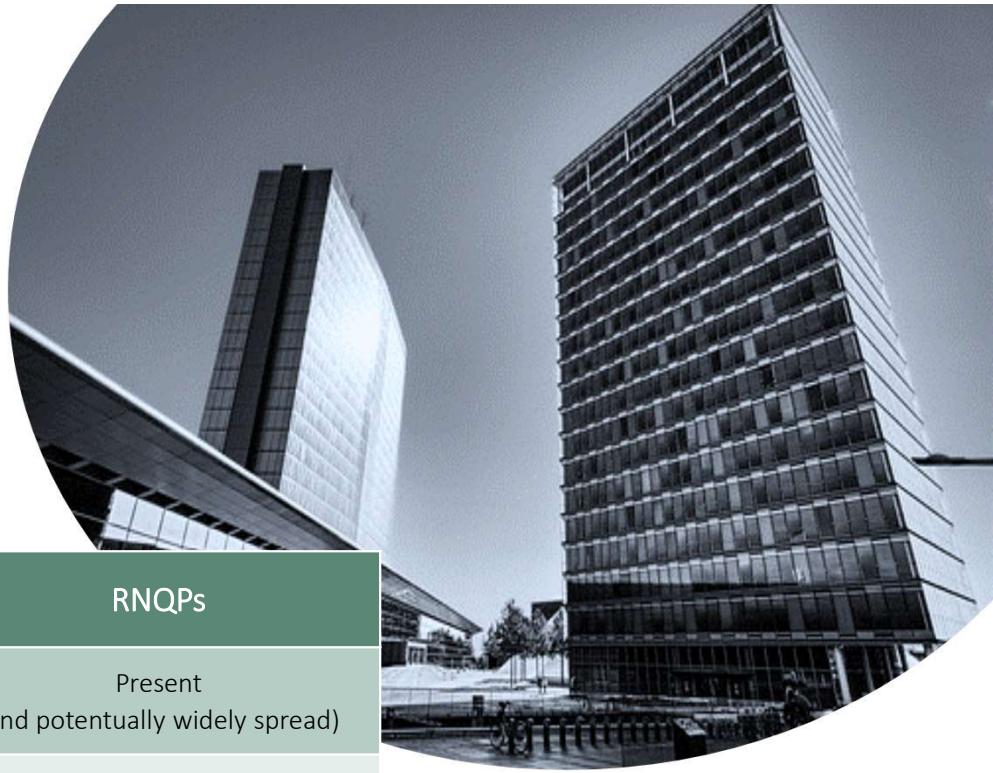
Cooperation with other laboratories

Participation in PTs per category:

	2017	2018	2019	2020	2021	2022
Bacteriology		1	3	1	3	2
Fungi					1	
Insects			1		1	1
Nematology		1	2	1	1	1
Virology	1	1	1	1	3	3
TOTAL	1	3	7	3*	9	7

* less participation due to the global pandemic

Classification of the potato viruses used in the PTs



Regulated Non-Quarantine Pests (RNQPs)

PVY and PLRV

PVX, PVA, PVS, (PVM)

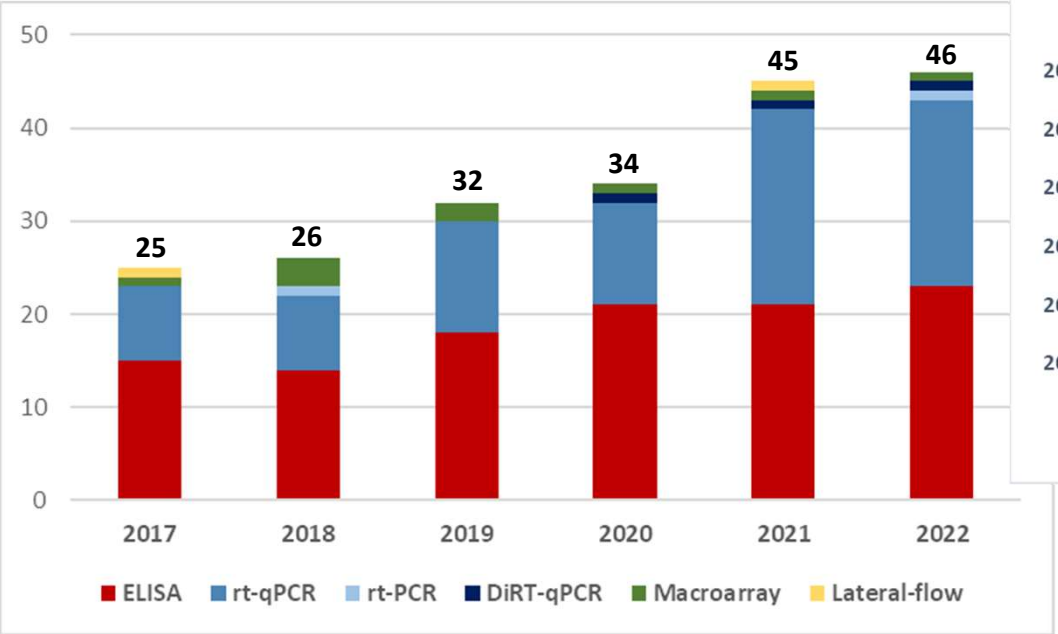
QPs and RNQPs at a glance:

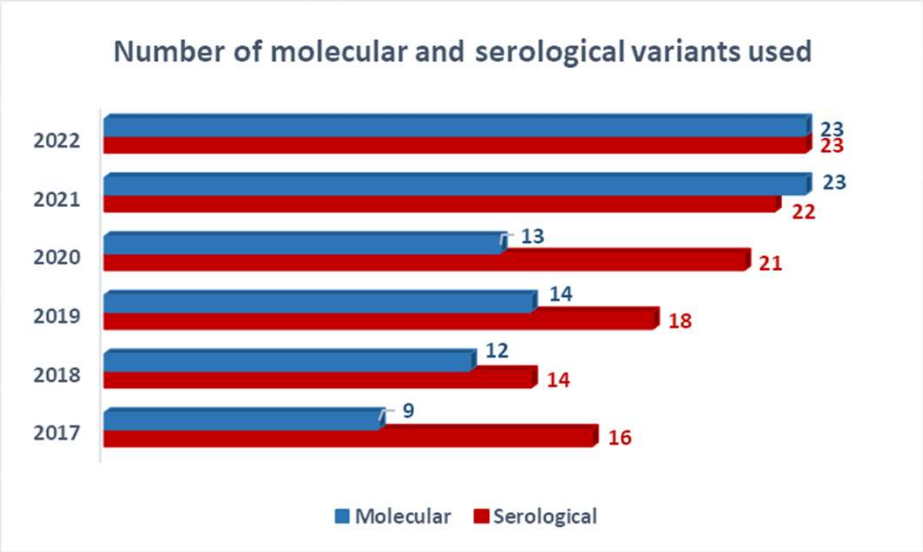
	Quarantine pests	RNQPs
Presence in the EU	Absent or limited distribution	Present (and potentually widely spread)
Phytosanitary measures	For all host plants	Only for seeds and planting material
Economical impact	Estimated	Known
Official measures	In case of presence : official monitoring (with aim of eradication or containment)	Official certification and control respecting the limits mentioned for seeds or planting material in the European legislation

Overview of participation in PTs for potato viruses

	2017	2018	2019	2020	2021	2022
Organizer	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (D)	Bayerische Landesanstalt für Landwirtschaft (D)	Landwirtschaftskammer Niedersachsen (D)	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (D)	Landwirtschaftskammer Niedersachsen (D)	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (D)
Sample manufacturing	Leibniz Institut DSMZ (D)	Leibniz Institut DSMZ (D)	Leibniz Institut DSMZ (D)	Leibniz Institut DSMZ (D)	Leibniz Institut DSMZ (D)	Leibniz Institut DSMZ (D)
Participating laboratories	19	18	21	22	31	31
Countries	7	8	11	11	18	16

PT for potato viruses: detection methods used by the participating laboratories





The use of molecular methods for potato virus detection has constantly increased over the last few years.

Applied test method in Luxembourg

In-house method with a multiplex real-time qPCR for:

1) **PVY – PLRV**
2) **PVX – PVA – PVS**

Real-time PCR performed with: **LightCycler 96**

Controls used:

Isolation controls: NIC, PIC
Amplification controls: PAC, NAC
Internal control: IC

Primers and probes selection:

PVY and PLRV: Boonham et al. (2009)
PVX: Agindotan et al. (2007); Mortimer-Jones et al. (2009)
PVA: Lacomme (2015)
PVS: Mortimer-Jones et al. (2009)

Results of the NRL Luxembourg in PTs for potato viruses

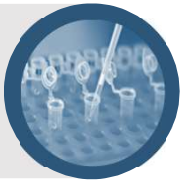
	2017	2018	2019	2020	2021	2022
Method	rt-qPCR	rt-qPCR	rt-qPCR	rt-qPCR	rt-qPCR	rt-qPCR
Number of implied laboratory technicians	2	2	2	2	3	2
Number of performance days (amplification)	2	3	3	2	2	2
C_t values for PVY	17.66	19.68	18.42	25.39	30.39	18.1
Result	**Diagnosis correct***	**Diagnosis correct**	**Diagnosis correct**	**Diagnosis correct**	**Diagnosis correct**	No final report

100% sensitivity
100% specificity
100 % accuracy

* All laboratories using rt-qPCR-method could not detect the used PVS isolate.

Why participating in PTs?

Aspects to implement the PTs in the laboratory routine



- Checking laboratory testing performance
- Illustrating the analytical quality of the laboratory
- Showing:
  - ✓ Process trends (f. ex. inaccuracy),
  - ✓ Relation to linearity, specificity, sensitivity, interferences and calibration characteristics

➢ **Confidence in laboratory performance is essential for laboratories and their customers (including regulatory authorities)**

- ✓ Quality control
- ✓ Frequency: annual
- ✓ Reasonable costs
- ✓ Reliable partner to obtain the PT material
- ✓ Reference material for further testing (calibration)

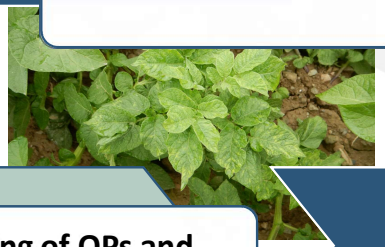
Participation of the Luxembourgish NRL in PTs for potato viruses

Seed potato production in Luxembourg including a quality assurance system

Meristem culture ➔ Mini-tuber production

Seed potato production

Field control by the official body



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Testing of QPs and RNQPs in the laboratory

Post control field

Quality assurance system in the Luxembourgish NRL

some examples (non-exhaustive list)

Quality assurance

- ✓ Documentation *(QM, SOPs)*
- ✓ Data evaluation
- ✓ Corrective actions
- ✓ Sustainable education of the staff
- ✓ …

Laboratory standardization

- ✓ Reference system *(reference material)*
- ✓ Traceability procedure *(qPCR efficiency analysis)*
- ✓ Verification procedure *(participation in PTs)*

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Thank you for your attention

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