Over 10 years' experience in potato virus proficiency tests as external proof of diagnostic laboratory competence

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EPPO Conference on Diagnostics of Plant Pests • AGES Vienna • 22-24 April 2026





Landwirtschaftskammer Niedersachsen

MV Mecklenburg-Vorpommern
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History of the proficiency test for potato viruses

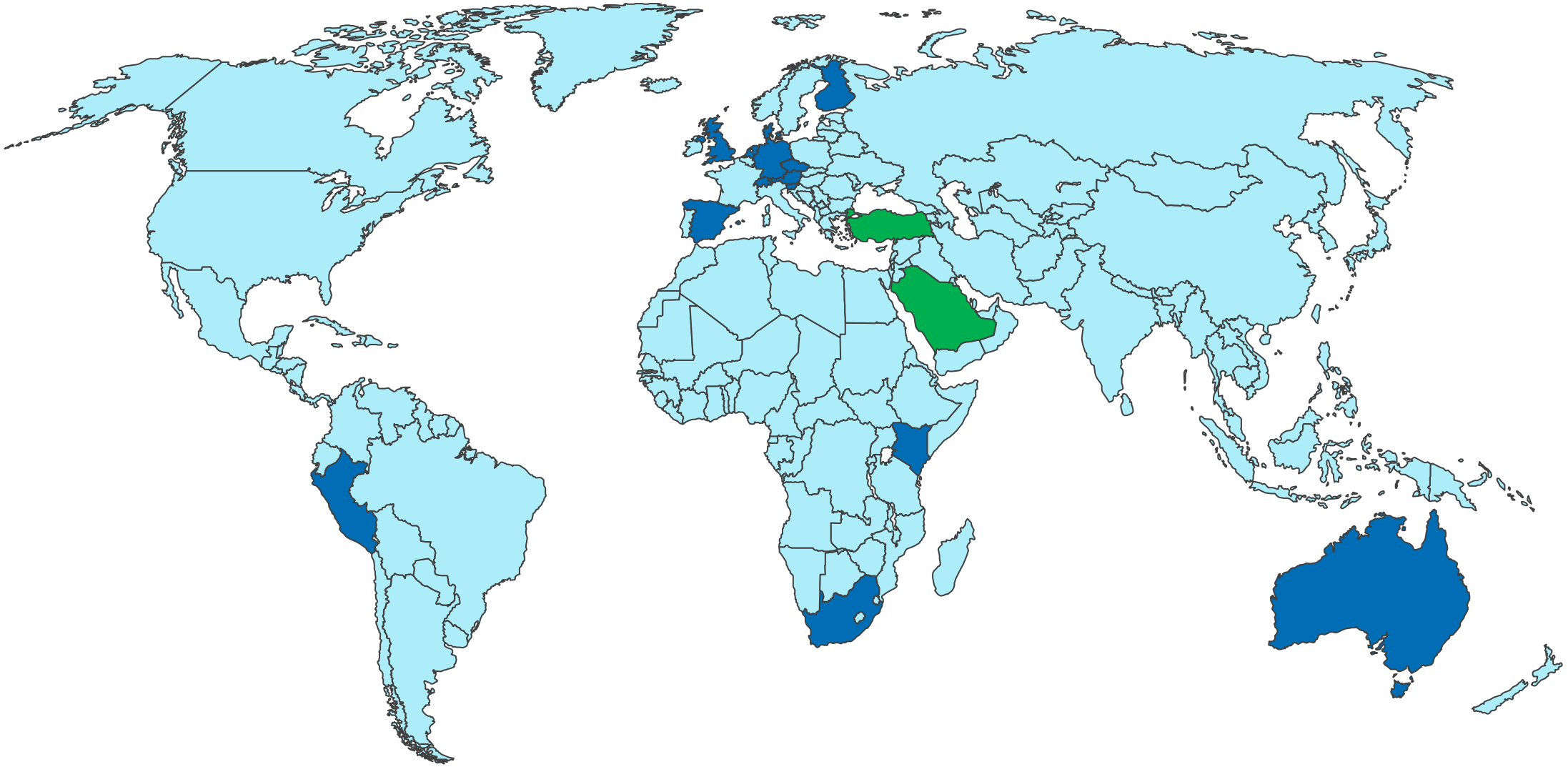
- In accordance with the German Seed Potato Regulation (PflKartV), seed potatoes are usually tested for PVX, PVY, PLRV, PVM, PVA and PVS
- The requirement to participate in a PT stems from the ISO 17025 accreditation
- Initiated in 2010 by three national laboratories: Lower Saxony, Mecklenburg, Bavaria
- In the early years, the sample material consisted of fresh leaves
- Results were not reproduceable and PT not accepted by auditors
- Since 2014 DSMZ as co-organizer for sample production and shipment

Samples/virus isolates used in 2025

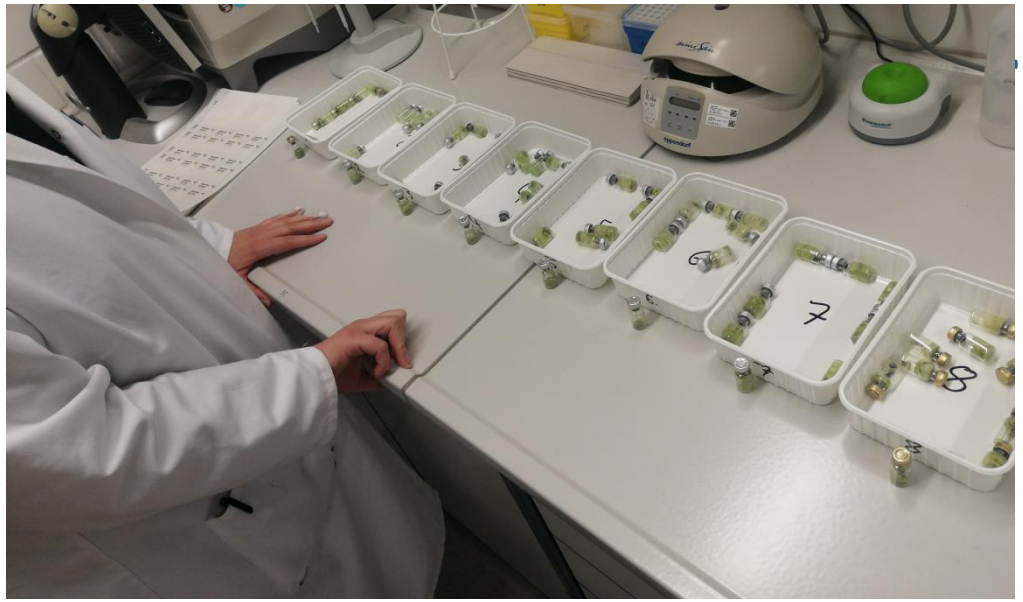
Sample 2025	Virus	Acc. No. DSMZ (GenBank)	Origin	Host
1	PVA	PV-0535 (MW197135)	Germany, BBA	*Nicotiana occidentalis* P1
2	PLRV	PV-0842 (MZ202339)	Germany, BBA	*Physalis floridana*
3	PVS	PV-1327 (OM471986)	Germany, Bioland	*Chenopodium quinoa*
4	PVM, PVY, PVX	W24-092	Germany, LALLF	potato
5	PVM	PV-0273 (MW582794)	Germany, BBA	*Nicotiana occidentalis* P1
6	PVX	W24-091	Germany, LALLF	potato
7	healthy	-	Germany, DSMZ	potato ‘Amado’
8	healthy	-	Germany, DSMZ	potato ‘Amado’

- W-Numbers are well characterized field samples, temporarily available
- All PV-numbers are permanent DSMZ reference isolates
- Sequences available at NCBI GenBank
- Prestesting done by ELISA (DSMZ) and RT-qPCR (LALLF)

2025: 39 participants from 18 countries with 59 sets of samples



Anonymization and participants



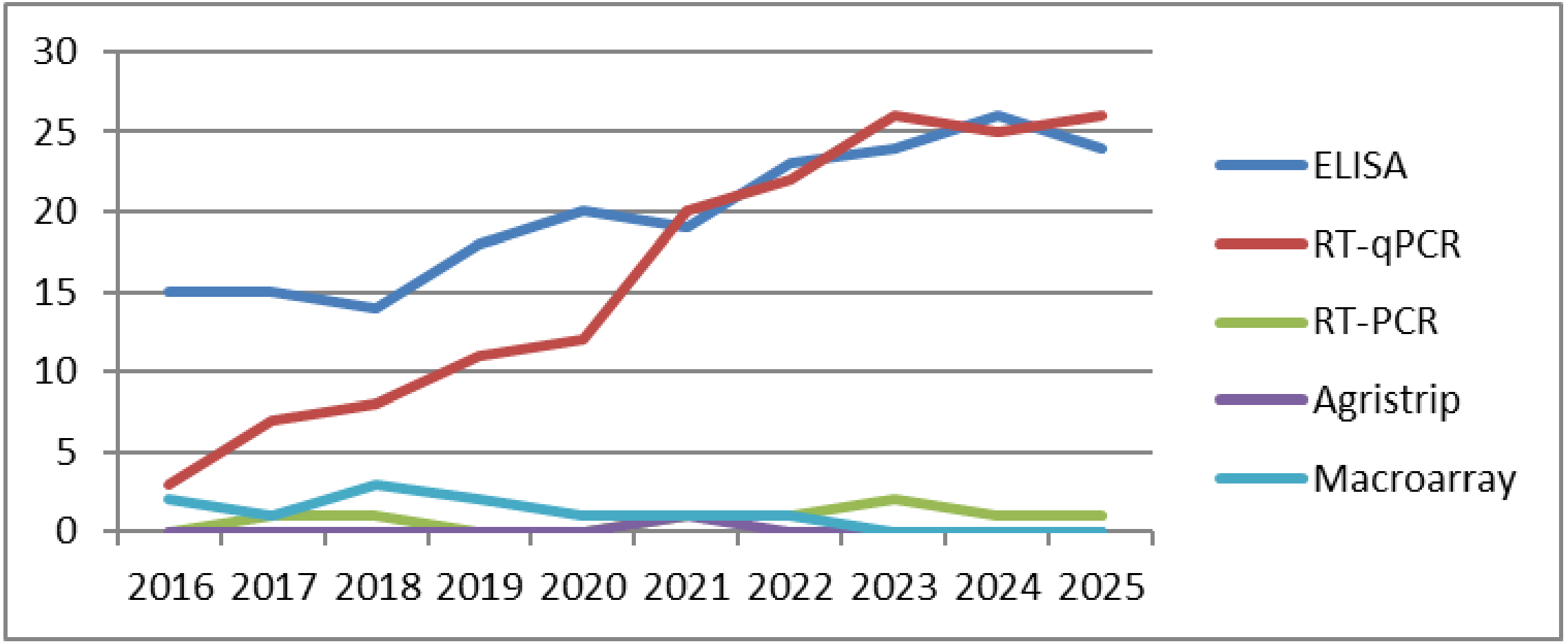
Country 2025	Laboratories	Sample sets
Germany	16	24
Luxembourg	1	2
Slovenia	1	2
Denmark	1	1
Austria	1	1
Finland	2	2
Netherlands	1	2
Czech Republic	1	1
Switzerland	2	3
Scotland	2	4
Saudi Arabia	3	3
Turkey	1	1
England	2	4
South Afrika	1	2
Australia	1	1
Peru	1	2
Kenya	1	2
Spain	1	2
∑ Countries = 18	**39**	**59**

Methods applied

Method	Sample sets analysed									
	2016	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**	**2024**	**2025**
ELISA	15	15	14	18	20	19	23	24	26	24
RT-qPCR	3	7	8	11	12	20	22	26	25	26
RT-PCR	0	1	1	0	0	1	1	2	1	1
Agristrip	0	0	0	0	0	1	0	0	0	0
Macroarray	2	1	3	2	1	1	1	0	0	0
∑	**20**	**24**	**26**	**31**	**33**	**42**	**47**	**52**	**52**	**51**

- **2025: results for 8 sets are pending**
- **RT-qPCR: Only 15 out of 26 laboratories test for all viruses (PVS, PVX, and PVM are often omitted)**
- **ELISA: Only one lab does not test for all viruses**

Methods used



Origin of kits/methods

Supplier ELISA	Bioreba	Agdia	DSMZ	Prime	SASA	Loewe
Sets analysed	16	1	4	2	1	1

RT-qPCR	Bioreba kit	published /in house
Sets analysed	4	22

Sample results

Ergebniseingang	23.02.2026	11.02.2026	11.02.2026	17.02.2026	24.02.2026	13.02.2026	21.01.2026	21.01.2026	26.01.2026	26.01.2026	23.02.2026
Methode	ELISA	RT-qPCR	RT-qPCR	RT-qPCR	ELISA	ELISA	ELISA	ELISA	ELISA	ELISA	ELISA
Quelle	Bioreba	several	several	Pastrik et al.	Bioreba/DSMZ	DSMZ	Bioreba	Bioreba	Bioreba	Bioreba	Bioreba/Prime
Probe/Teilnehmer	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
1 PVA	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	PVY f-pos
2 PLRV	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
3 PVS	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
4 PVM, PVY, PVX	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
5 PVM	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
6 PVX	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
7 Kartoffel gesund	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
8 Kartoffel gesund	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺

Ergebniseingang	19.02.2026	23.02.2026	23.02.2026	01.01.2026	23.02.2026	23.02.2026	17.02.2026	17.02.2026	23.02.2026	20.02.2026	20.02.2026
Methode	RT-qPCR	RT-qPCR	ELISA	RT-qPCR	ELISA	RT-qPCR	RT-qPCR	ELISA	RT-PCR	ELISA	RT-qPCR
Quelle	several	Schumpp 202	Loewe	several	Bioreba	Bioreba	unpublished	Bioreba	several	Agdia	unknown
Probe/Teilnehmer	**AS**	**AT**	**AU**	**AV**	**AW**	**AX**	**AY**	**AZ**	**BA**	**BB**	**BC**
1 PVA	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
2 PLRV	☺	☺	☺	PVY f-pos	☺	☺	☺	☺	☺	☺	☺
3 PVS	☺	☺	☺	☺	☺	☺	PVY f-pos	☺	☺	☺	☺
4 PVM, PVY, PVX	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	PVY f-neg
5 PVM	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
6 PVX	☺	☺	☺	☺	☺	☺	☺	☺	PVX f-neg	☺	☺
7 Kartoffel gesund	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺
8 Kartoffel gesund	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺	☺

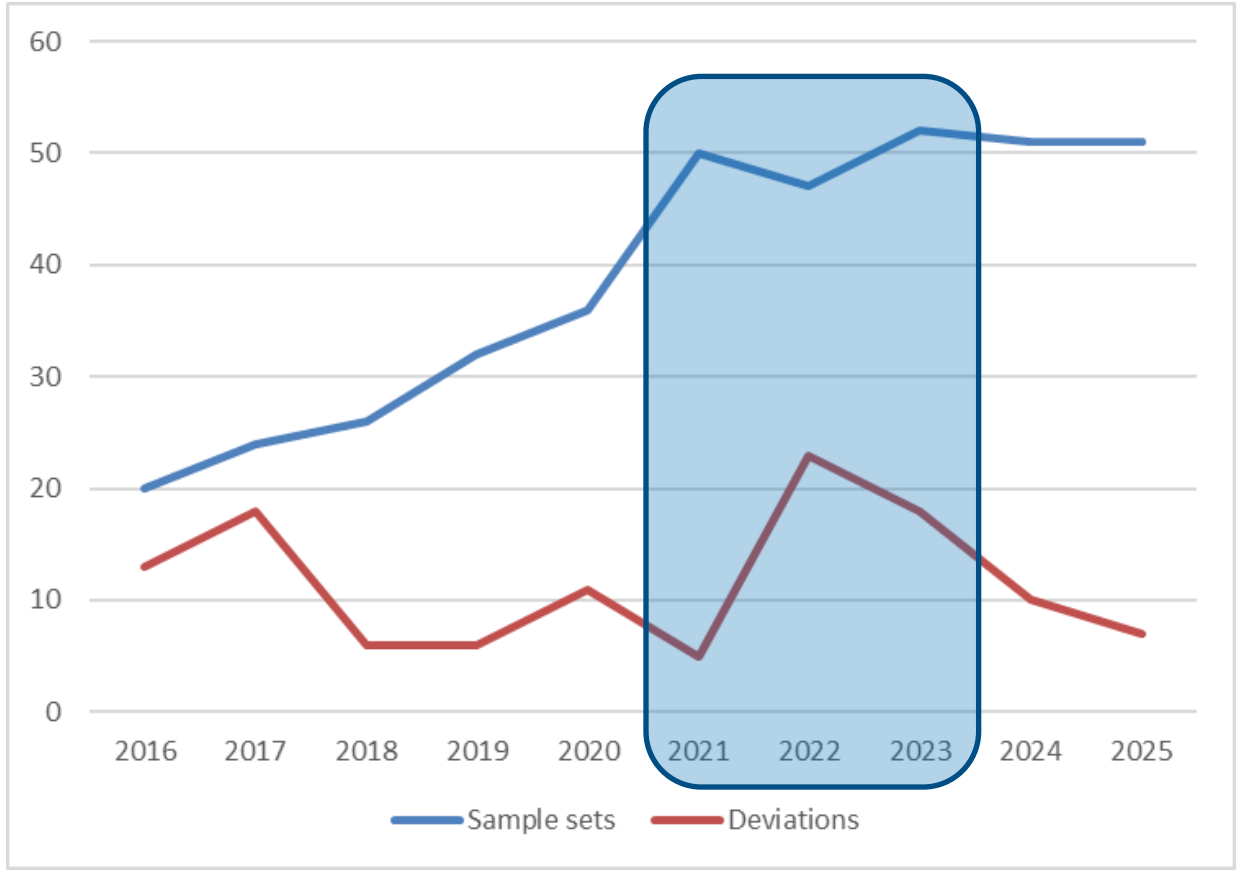
In 2025: Only 7 deviations in 51 sample sets analysed (in total 408 samples, corresponding to ca. 2,400 individual tests)

Virus	Deviations by method			
	ELISA	ELISA	RT-qPCR	RT-qPCR
	false negative	false positive	false negative	false positive
∑	0	1	2	4
PLRV				1
PVY		1	1	3*
PVM				
PVA				
PVX			1*	
PVS				

*RT-PCR

*3 different batches

Deviations over the years



	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
Sample sets	20	24	26	32	36	50	47	52	51	51
Deviations	13	18	6	6	11	5	23	18	10	7

First-time participants

Ergebniseingang	17.02.2023	09.02.2023	26.01.2023	17.01.2023	17.01.2023	17.01.2023	1
Methode	RT-qPCR	RT-qPCR	ELISA	ELISA	Macroarray	RT-qPCR	RT
Quelle	Bioreba/Kit	unpublished	Sediag	Bioreba	Bioreba	Bioreba/Kit	Fu
Probe/Teilnehmer	A	B	C	D	E	F	
1 Kartoffel gesund	PVX f-pos	☺	☺	☺	☺	☺	
2 PVX	siehe 1)	☺	☺	☺	☺	☺	
3 PLRV, PVM, PVY, PVS	PVX f-pos	☺	☺	☺	☺	☺	
4 PVS	☺	☺	☺	☺	☺	☺	
5 PLRV, PVM	PVA f-pos	☺	☺	☺	☺	☺	
6 PVA	☺	☺	☺	☺	☺	☺	
7 PLRV, PVM, PVS	PVS f-neg	☺	☺	☺	☺	☺	
8 PVX	☺	☺	☺	☺	☺	☺	
	1) PLRV f-pos, PVY f-pos, PVS f-pos, PVM f-pos						

Using a kit does not guarantee accurate results

Differences in ct Values for the Bioreba RT-qPCR Kit

Bioreba RT-qPCR (ct)	PVY	PVM	PLRV	PVX	PVS	PVA
Anwender 1	17	14, 15	18	17, 22	16	16
Anwender 2	18	13, 14	19	14, 17	14	14
Anwender 3	25	25, 26	24	24, 25	21	23
Anwender 4	29	24, 26	21	22, 23	26	23

PVS variability and mismatches with primers/probe

- **In the PT in 2017 most labs using RT-qPCR missed PVS (PV-0756, belonging to the “Andean strain”)**
- **Those labs used the primer/probe combination published by Mortimer-Jones et al.(2009)**
- **These show numerous mismatches to Andean strain isolates including PV-0756**

Variant B: “Andean strain“

Variant A: “ordinary strain“

Summary and Conclusions

- What started as a personal initiative has grown into a successful, self-organized PT
- High international demand highlights the global shortage of PTs
- The number of labs testing tubers directly using RT-qPCR has increased, but ELISA use remains stable
- Deviations stable at a low level
- “Newcomers” from the early 2020s have gotten their problems under control
- Successful application and sensitivity in RT-qPCR varies greatly depending on the user/laboratory
- PTs help identify weaknesses in published methods (PVS RT-qPCR, Mortimer-Jones et al. 2009)

Thank you for your attention!

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Prestesting by ELISA (DSMZ) and RT-qPCR (LALLF)

A/B: 2 vials/batch tested

Sample 2025	Virus	ELISA (OD 2h A/B)	RT-qPCR (ct A/B)
1	PVA	1,5/1,6	11,8/11,7
2	PLRV	3,8/3,8	14,0/13,5
3	PVS	3,7/3,8	10,3/10,3
4	PVM, PVY, PVX	PVM: 1,5/1,5 PVY: 0,4/0,5 PVX: 2,3/2,5	PVM: 12,7/12,4 PVY: 13,4/12,4 PVX: 6,5/6,6
5	PVM	1,8/1,5	12,0/11,8
6	PVX	2,9/3,3	14,2/13,6
7	gesund	-	-
8	gesund	-	-

Detailed information on participants' false-positive results

- **ELISA PVY false positive: OD 0,29** **PVY true positive: OD 1,46**
- **RT-qPCR PLRV false positive: ct 28** **PLRV true positive: ct 15**
- **RT-qPCR PVY false positive: ct 27** **PVY true positive: ct 14**
- **RT-qPCR PVY false positive: ct 18** **PVY true positive: ct 16**
- **RT-qPCR PVY false positive: ct 35** **PVY true positive: ct 22**

Sample 2025	Virus	RT-qPCR pretesting (ct A/B)
2	PLRV	14,0/13,5
4	PVY	13,4/12,4