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From spiking to routine use: versatile applications of reference materials for Xylella fastidiosa diagnostics

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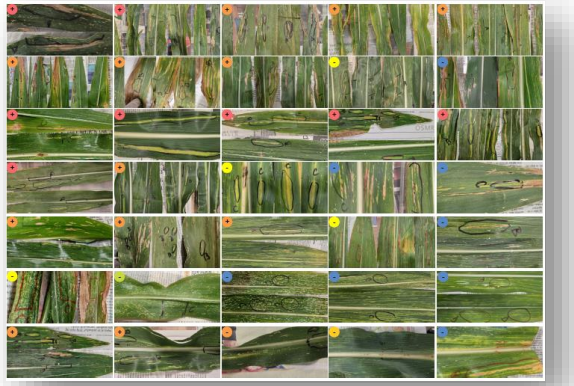
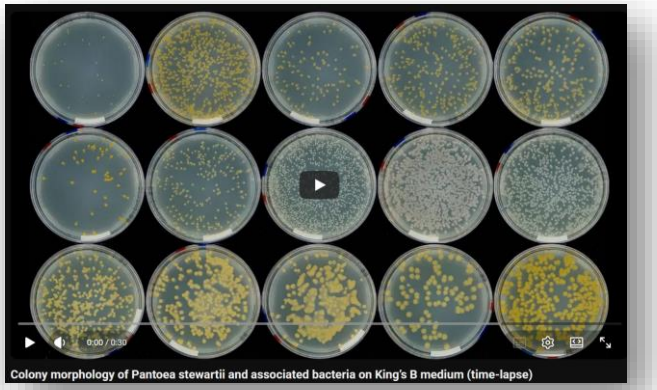
EPPO Conference on Diagnostics of Plant Pests
Recent developments and future trends
2026-04-22/24, Vienna (AT)

Reference materials

- Reference materials are **indispensable for reliable plant pest diagnostics**
- Reference material is defined as **material suitable for testing and diagnosis**, which is **documented** and **reliably expresses the characteristics for which it is selected (EPPO PM 7/76)**.



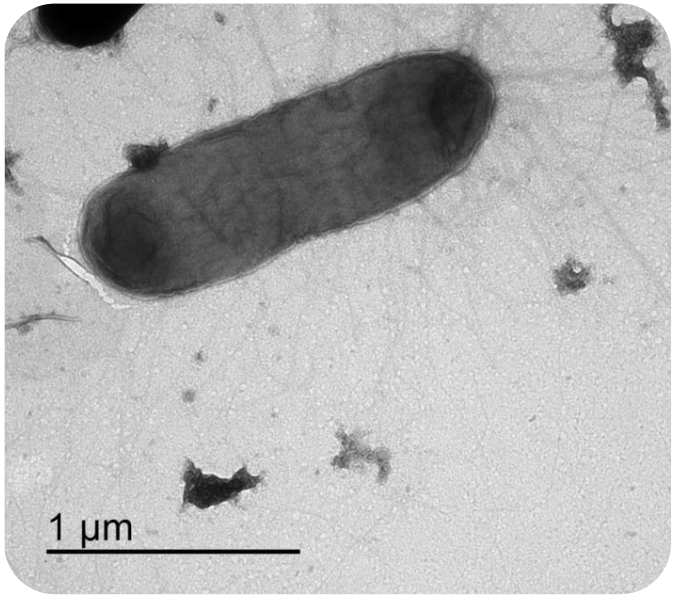


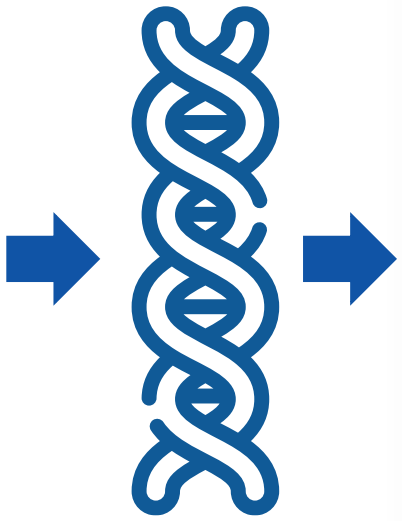
- **High-quality** reference materials require **substantial effort**
- A summary of **how to maximise their use across diagnostics**

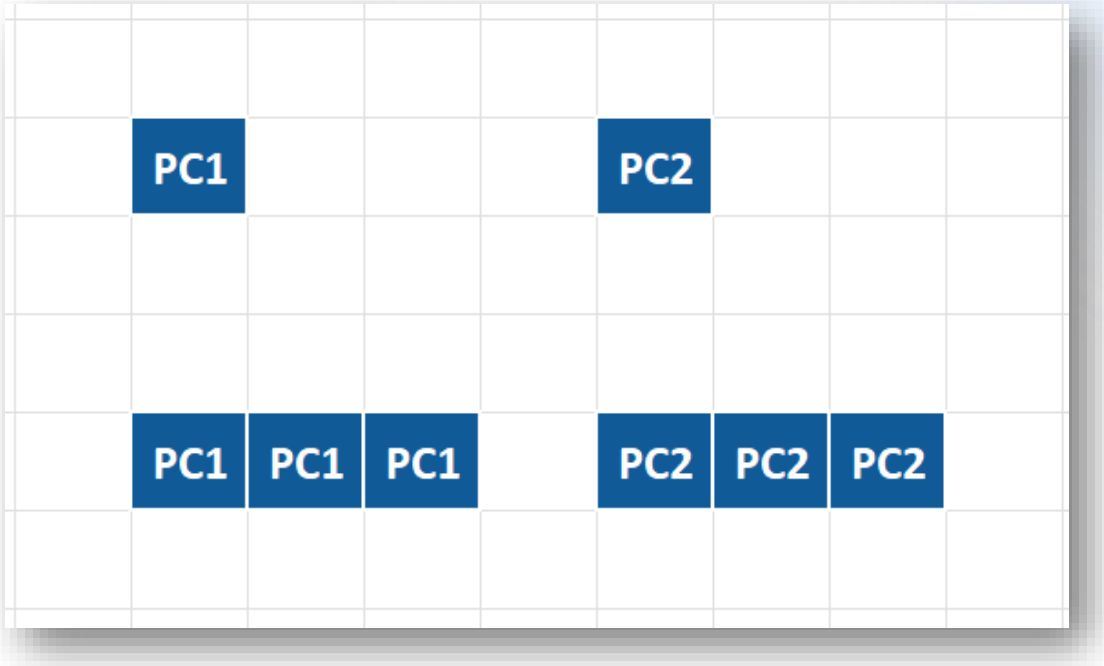
Pirc, M., & Dreo, T. (2026). https://doi.org/10.5281/zenodo.19626542; Matičič et al., (2026). https://doi.org/10.5281/zenodo.19593528.

Real-time positive amplification (PAC) controls

- used beyond validation → **embedded in routine diagnostics**
- DNA of the target organism at two mid range concentrations.



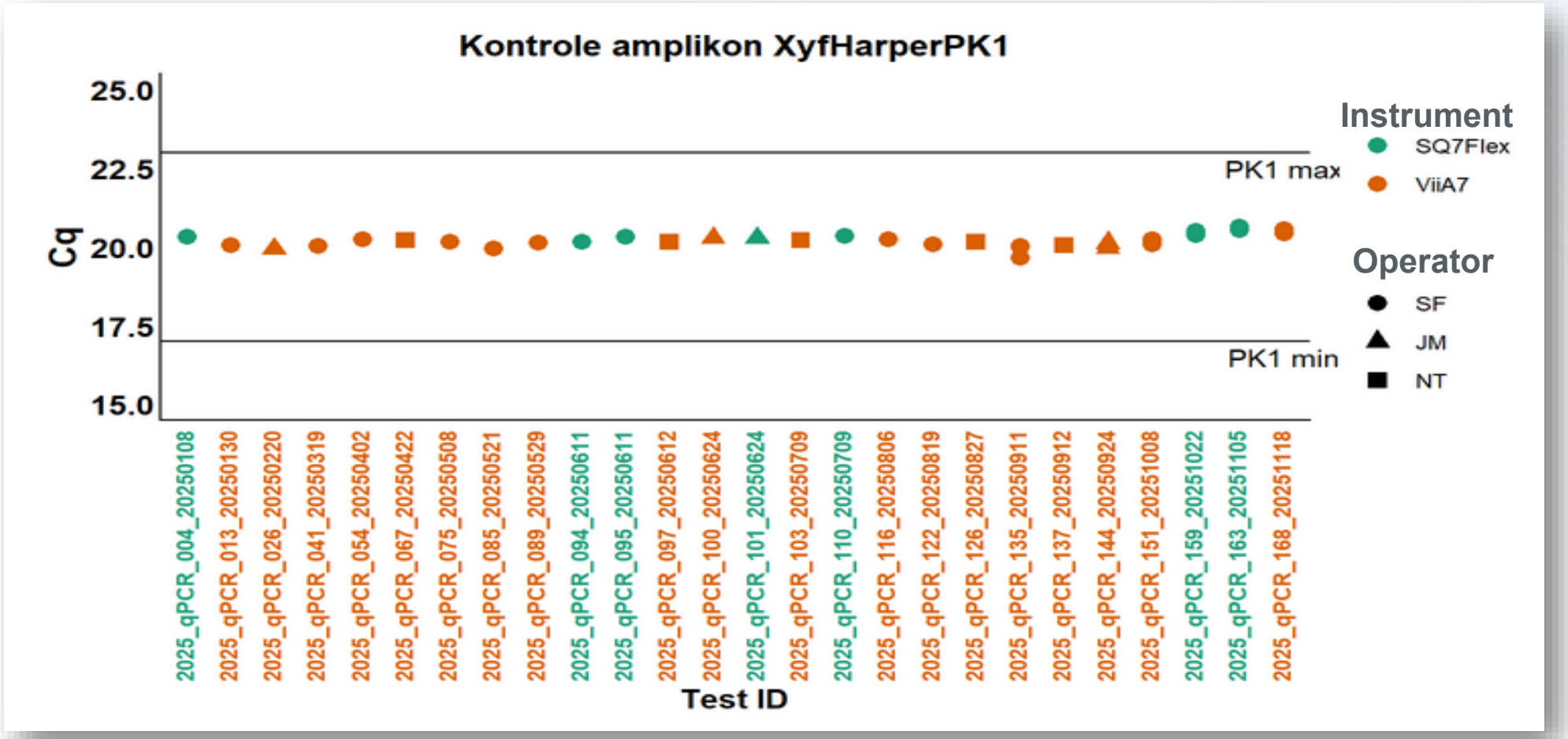




TEM of X: fastidosa by Magda Tušek Žnidarič, NIB.

Process monitoring in routine workflows

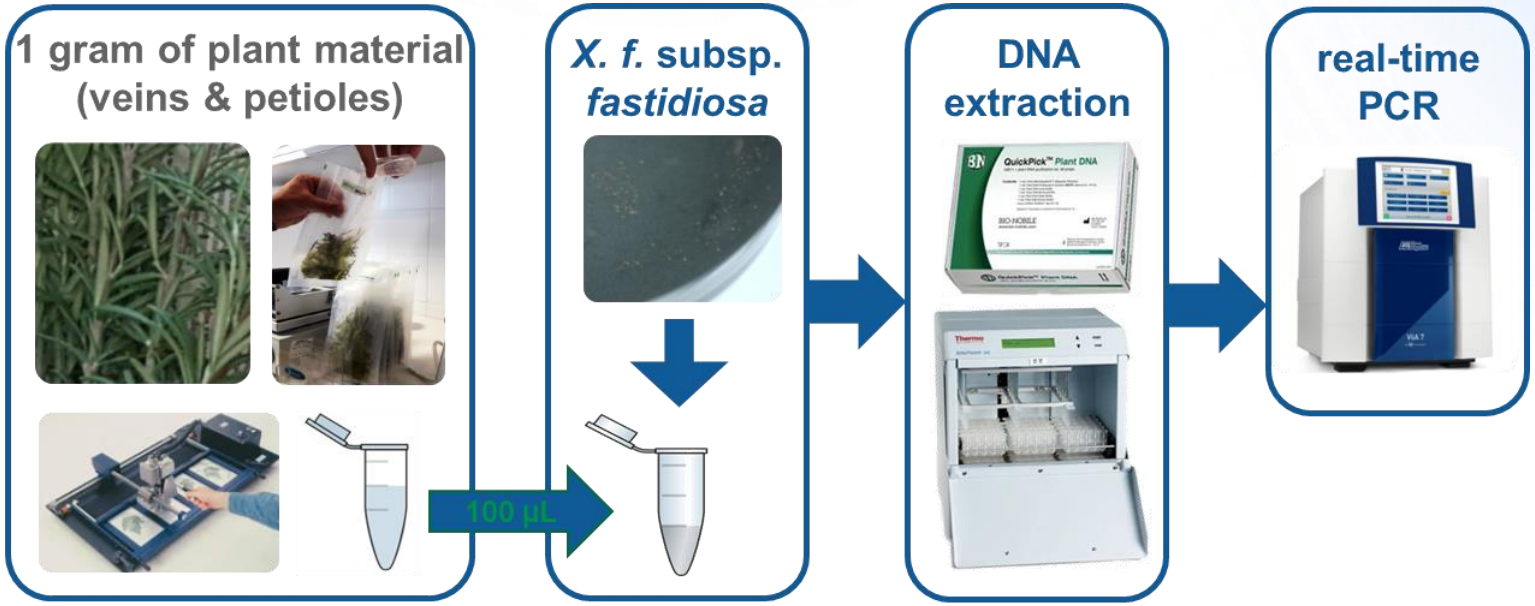
- process monitoring: real-time PCR



Positive NAC isolation control „PKIe“

- Used beyond validation → **embedded in routine diagnostics**
- **Process control: DNA extraction, molecular tests, matrix effect**

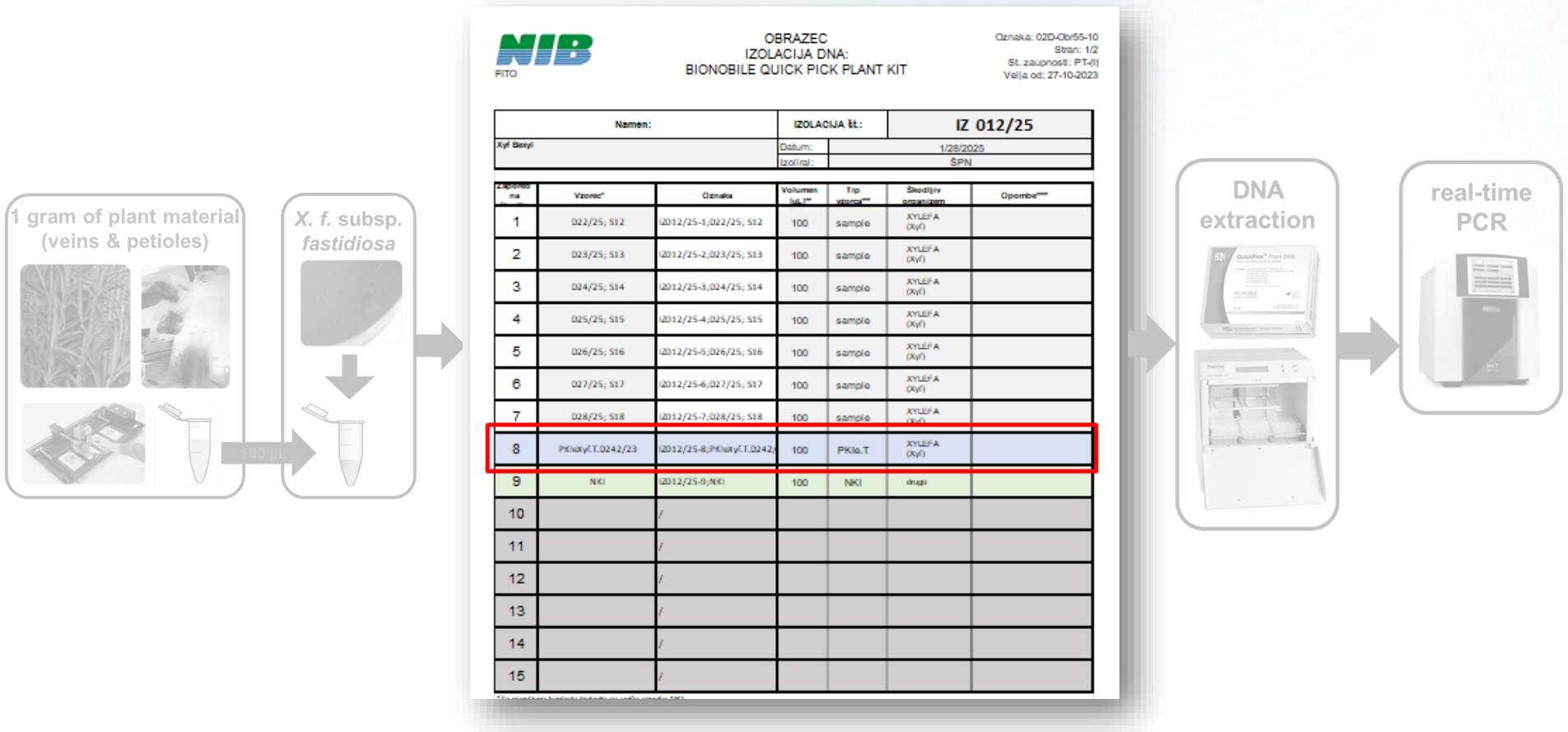
- **Since 2021**
- prepared in line with the **EPPO Standard PM 7/147** to ensure traceability and comparability



Photographs of instruments and kits by BIOREBA (Homex homogenizer), BIO-NOBILE (Quick Pick Plant Mini kit), Thermo Fisher Scientific (KingFisher mL) and Life Technologies (ViiA7).

One PIC used in each DNA extraction series

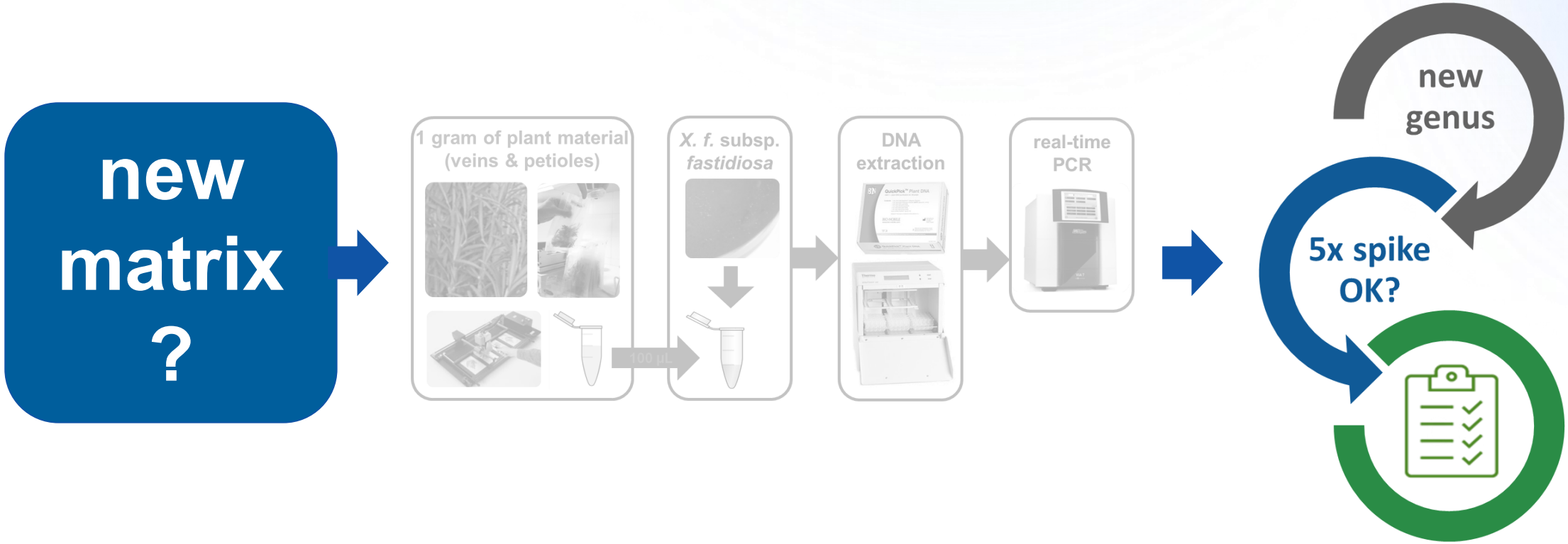
→ has to be positive for a valid test.



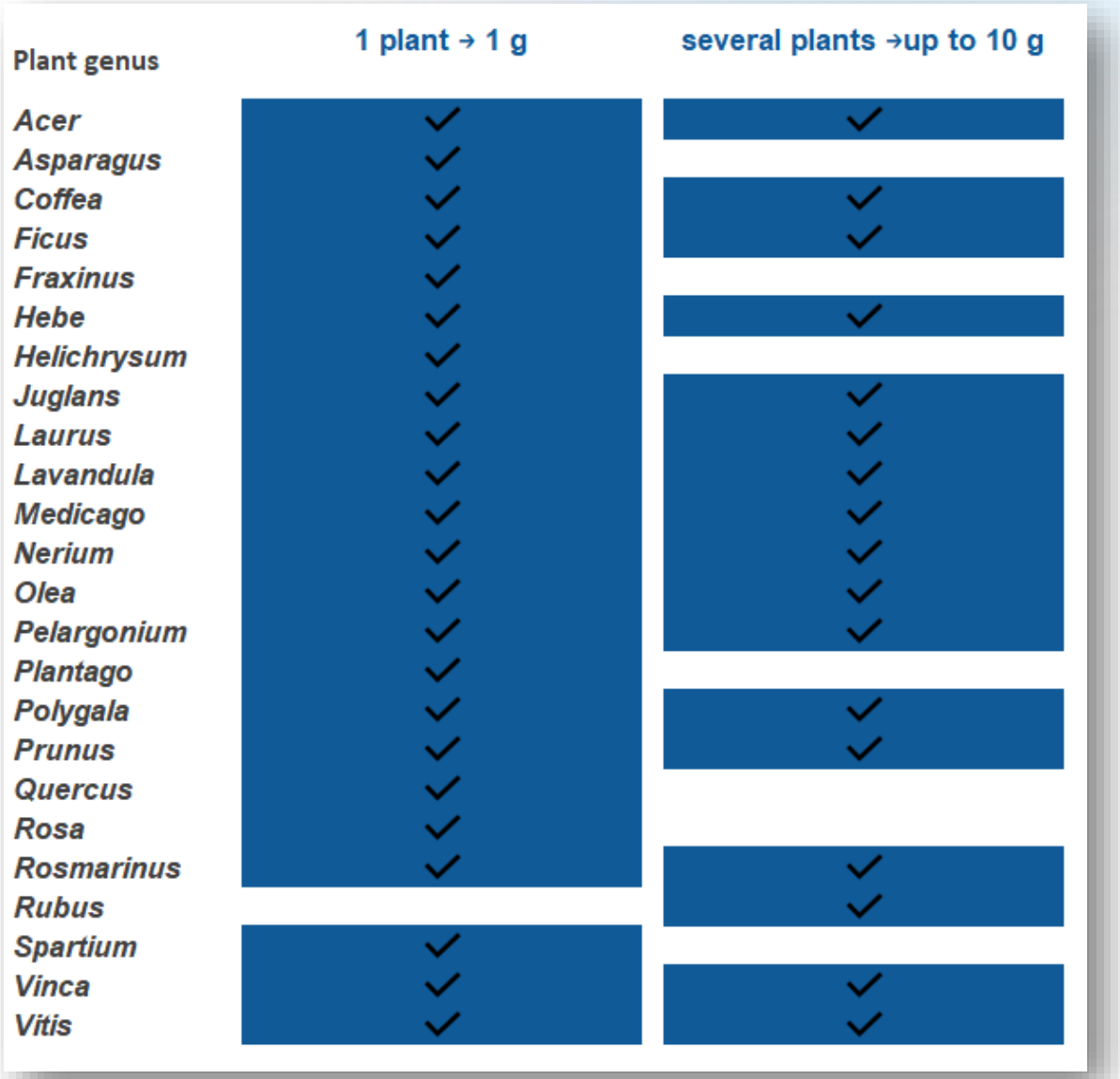
Validation of tests across diverse host plants: spiked samples

Xylella fastidiosa can infect 500+ plant species across 300+ plant genera.

- **Dynamic validation of matrices**



→ List of validated matrices (ISO/IEC 17025)



Plant genus	1 plant → 1 g	several plants →up to 10 g
Acer	✓	✓
Asparagus	✓	
Coffea	✓	✓
Ficus	✓	✓
Fraxinus	✓	
Hebe	✓	✓
Helichrysum	✓	
Juglans	✓	✓
Laurus	✓	✓
Lavandula	✓	✓
Medicago	✓	✓
Nerium	✓	✓
Olea	✓	✓
Pelargonium	✓	✓
Plantago	✓	
Polygala	✓	✓
Prunus	✓	✓
Quercus	✓	
Rosa	✓	
Rosmarinus	✓	✓
Rubus		✓
Spartium	✓	
Vinca	✓	✓
Vitis	✓	✓

Identification of diagnostic gaps

- % positive reactions → **DNA recovery depending on matrix**

Plant genus	undiluted	1:10 diluted	Plant genus	undiluted	1:10 diluted
Nerium	100.0%	100.0%	*Spartium*	100.0%	100.0%
Rosmarinus	100.0%	100.0%	*Vinca*	100.0%	100.0%
Lavandula	100.0%	90.9%	*Rosa*	100.0%	100.0%
Olea	100.0%	94.1%	*Acer*	100.0%	90.0%
Asparagus	100.0%	100.0%	*Vitis*	100.0%	100.0%
Ficus	100.0%	100.0%	*Hebe*	100.0%	100.0%
Juglans	100.0%	100.0%	*Coffea*	100.0%	100.0%
Laureus	100.0%	100.0%	*Prunus*	100.0%	92.1%
Medicago	100.0%	100.0%	All*	100.0%	96.6%
Polygala	100.0%	100.0%	Other**	100.0%	100.0%

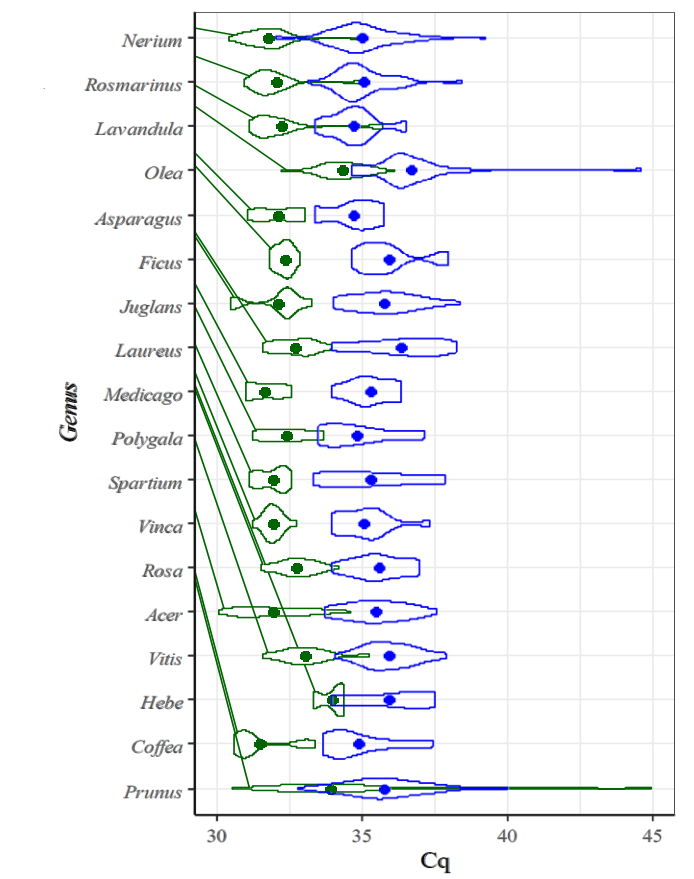
** data shown is based on analysis of from minimum five (5) to forty-eight (48) spiked samples per genus; ** 27 spiked samples of the following genera (< five/genus): Acacia, Amaranthus, Callistemon, Cercis, Chenopodium, Citrus, Ginko, Grevillea, Helichrysum, Heliotropium, Hydrangea, Liquidambar, Lonicera, Morus, Myrtus, Pelargonium, Quercus, Rhamnus, Streptocarpus, Vaccinium.*

Identification of diagnostic gaps

- absolute quantification VS Cq → **matrix dependent DNA recovery**

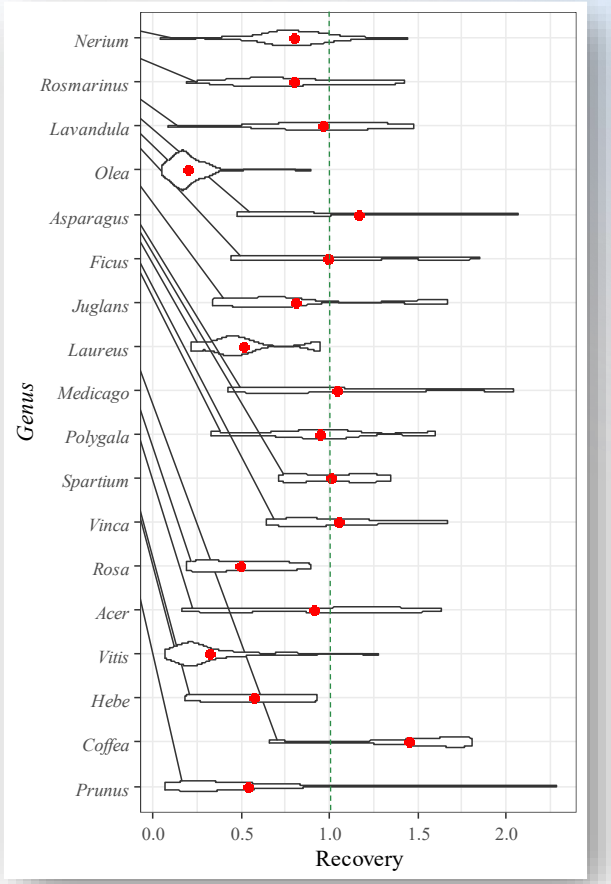
Cq values and inhibition

undiluted
1:10 diluted



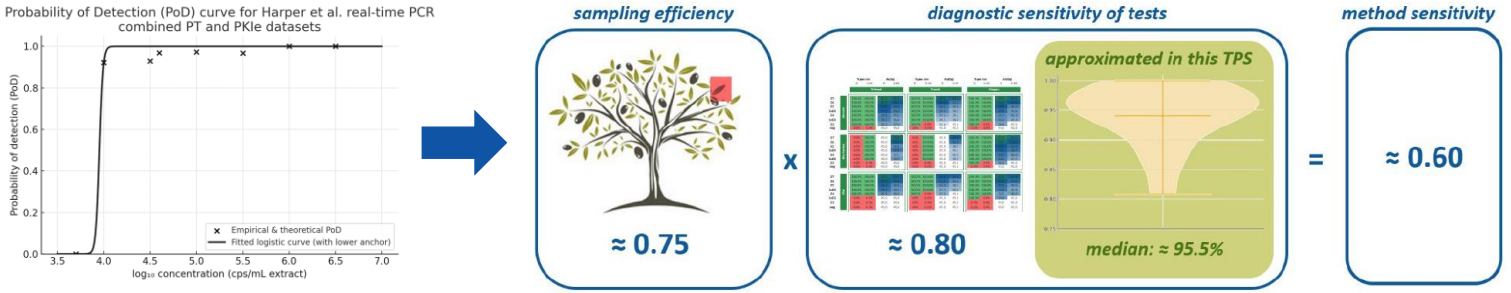
Recovery

- N = 320
- % [conc (no matrix) / conc (matrix)]
- from 4 to 228 %
- median recovery of 54 % (N = 311)



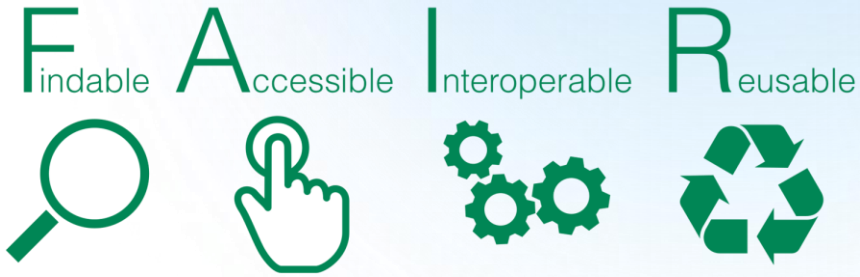
Informing surveying strategies

- % positive reactions → an **approximation of the diagnostic sensitivity**
- a parameter in **statistically supported survey planning**
  - a digital PCR TPS (BeXyl)



  - real-time PCR

Source	Type of material	N(rxns)	≈ DSe
Internal PKIe controls	Spiked (PICs, samples)	1818	0.97
ILS (DNA only)	Purified DNA (various subspp.)	312	0.97
ILS (full workflow)	Plant tissue samples (with extraction)	286 + 78 -	0.94



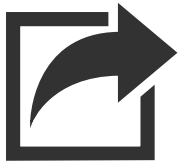
- **Cross-laboratory comparison of matrix effects**: identify problematic hosts and extraction biases.
- **Shared benchmarks** for reference materials: comparable Cq values, recovery rates and performance ranges.
- **Data-driven survey design**: use real detection probabilities instead of assumptions.
- **Faster validation** for new matrices or hosts: **reuse existing datasets** instead of repeating experiments.
- **Transparent and reusable diagnostic evidence**: support accreditation, audits and method extension.

Conclusions

Reference materials are not static controls but versatile tools that support the full diagnostic workflow. They enable:

- High-quality **reference materials** are essential for reliable diagnostics
- Their value extends across the **entire diagnostic workflow**
- They reveal **matrix-dependent effects** and **diagnostic variability**
- Their impact increases through **long-term collection and accumulated experience**

Future directions



- **Expand** reference collections
- **Build** long-term experience
- **Extend** to less frequently tested organisms and non-molecular methods
- **Enable** multi-laboratory data sharing

Acknowledgements

REPUBLIKA SLOVENIJA
MINISTRSTVO ZA KMETIJSTVO, GOZDARSTVO IN PREHRANO

UPRAVA REPUBLIKE SLOVENIJE ZA VARNO HRANO, VETERINARSTVO IN VARSTVO RASTLIN

- *Plant Health Tasks*

ARIS Javna agencija za znanstvenoraziskovalno in inovacijsko dejavnost Republike Slovenije

- *Research programmes Biotechnology and Systems Biology P4-0165 in P4-0463*

BEXYL
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EURL
European Union Reference Laboratory for **Plant Pathogenic Bacteria**

Co-funded by the European Union

NIB