

Diagnostic Assay Validation Network (DAVN)

EPPO Conference on Diagnostics of Plant Pests

Recent developments and future trends

2026-04-22, Vienna (AT)

Diagnostic Assay Validation Network (DAVN)

Development • Validation • Application



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Project funded by USDA- NIFA

Award No. 2022-68013-36537 (3-1-2022 to 2-28-2025)

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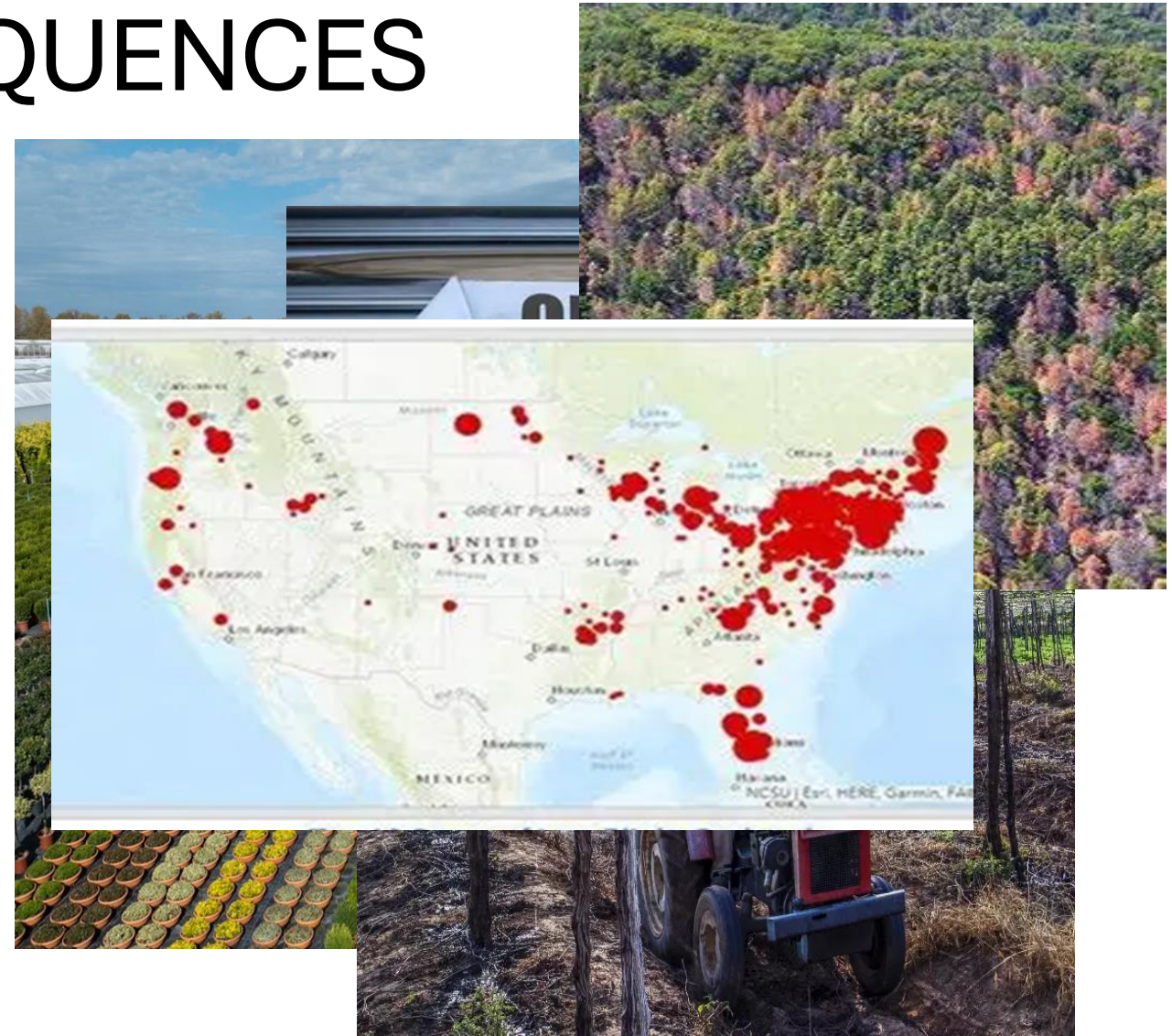
Co-PIs – Carrie Harmon (UFL), Poonam Sharma (OSU)



Why do we validate diagnostic tests?

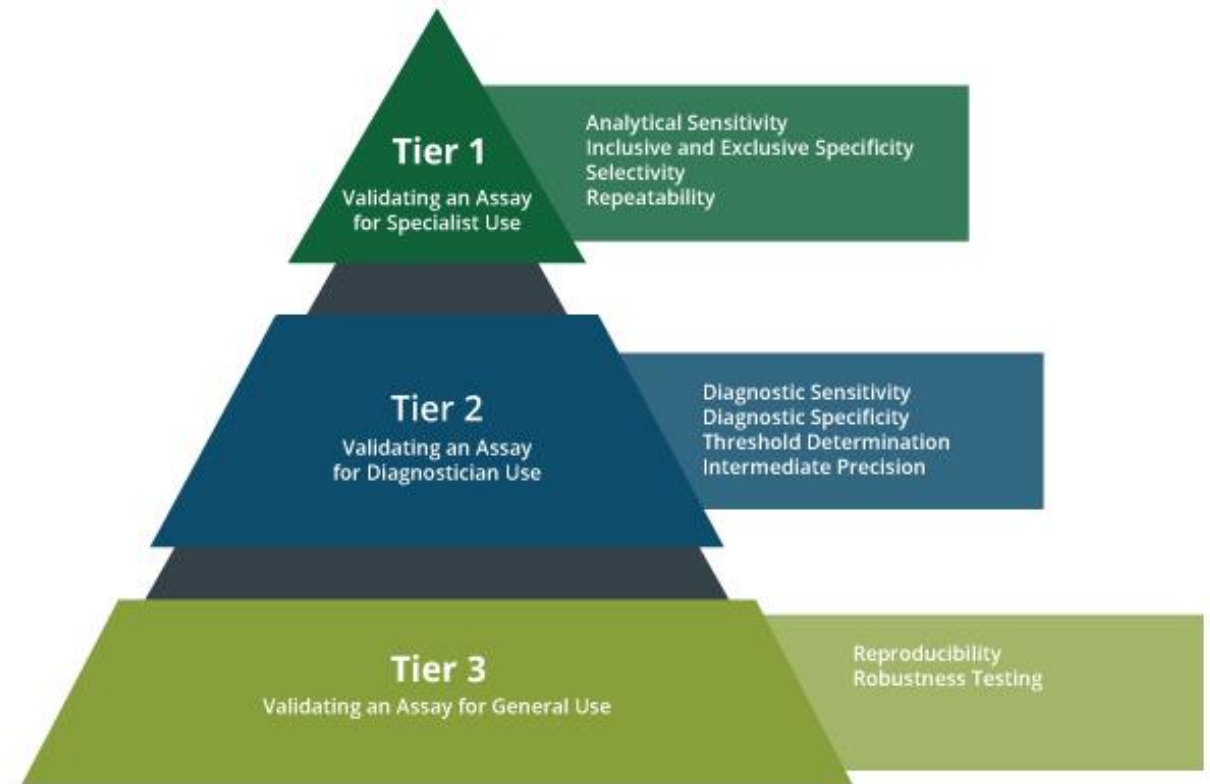
CONSEQUENCES

- Results of False Positives (lack of specificity)
- Results of False Negatives (lack of sensitivity)
- Results of lack of test reproducibility and robustness



Test fitness for purpose – who uses it and when?

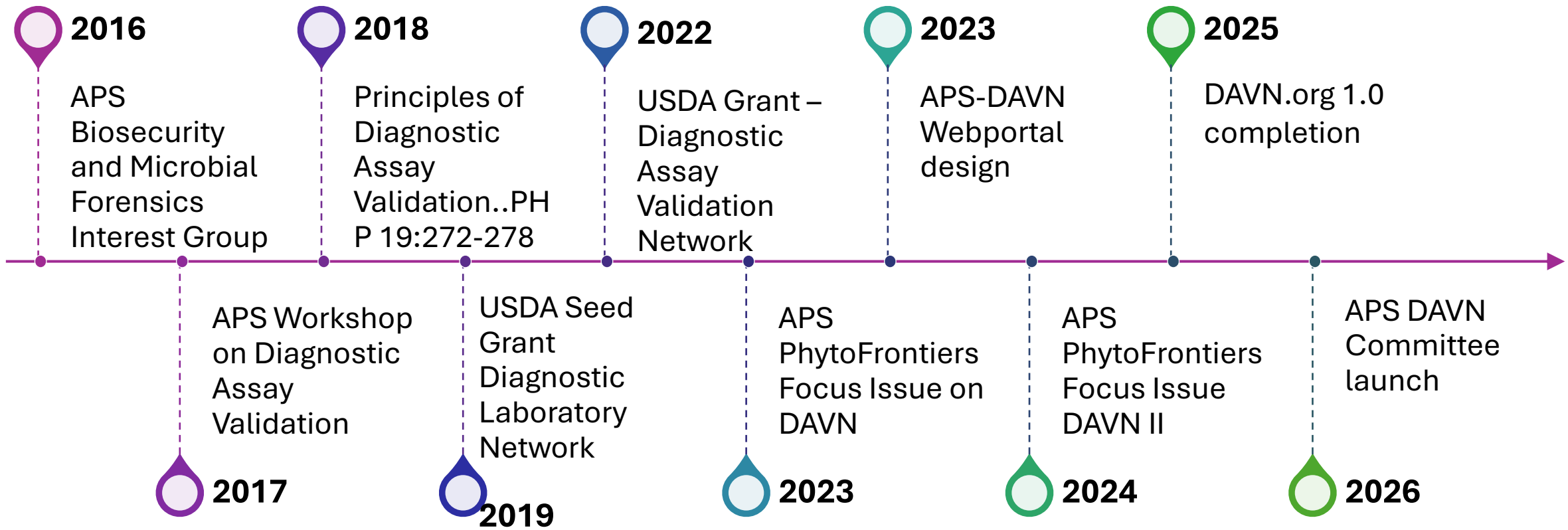
- Research –tests developed by researchers for taxa id
- Regulatory screening tests show ‘freedom from’ (expert lab)
- General surveillance testing for absence/presence of a suite of pests and pathogen of concern (many labs)
- Diagnostic test of infected material (diagnostic lab)
- Delimiting survey (specificity to genus level many labs)
- Regulatory test to confirm and respond to new outbreak



Groth-Helms, et al., 2023

<https://doi.org/10.1094/PHYTOFR-05-22-0059-FI>

History and Future of DAVN





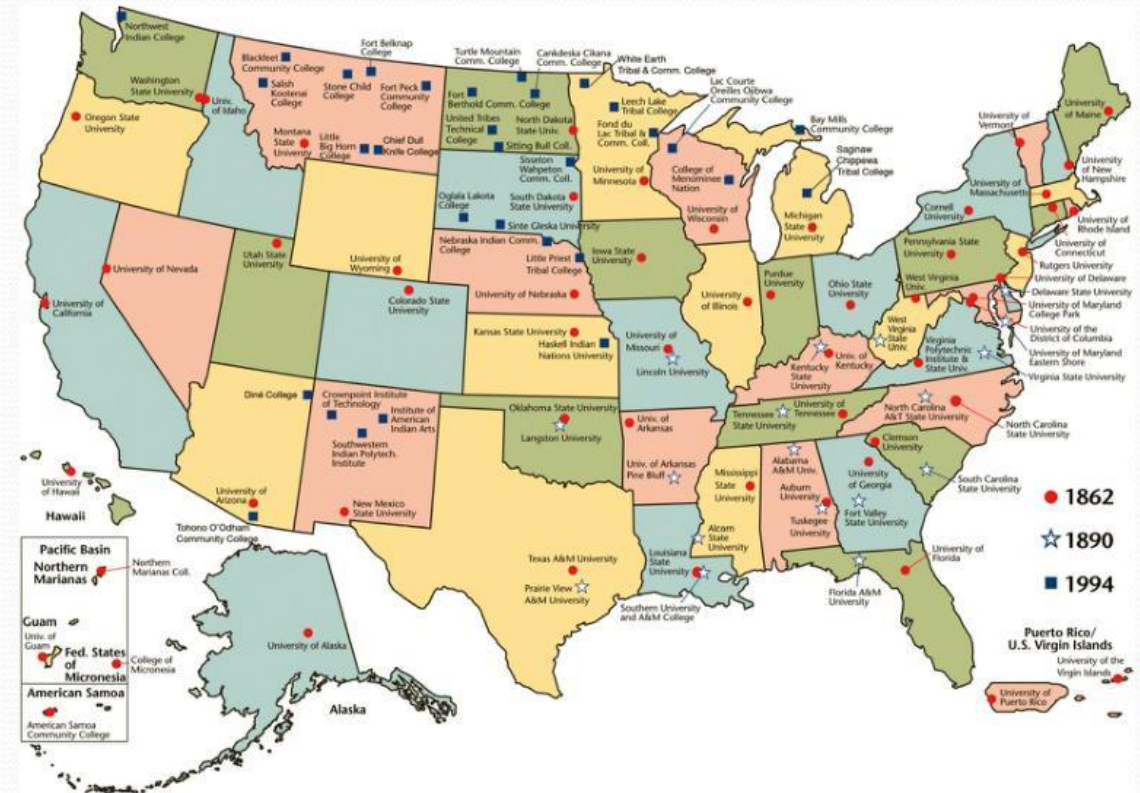
Complexity in the US

Challenges and Opportunities



Land Grant Universities

Land Grant Colleges and Universities

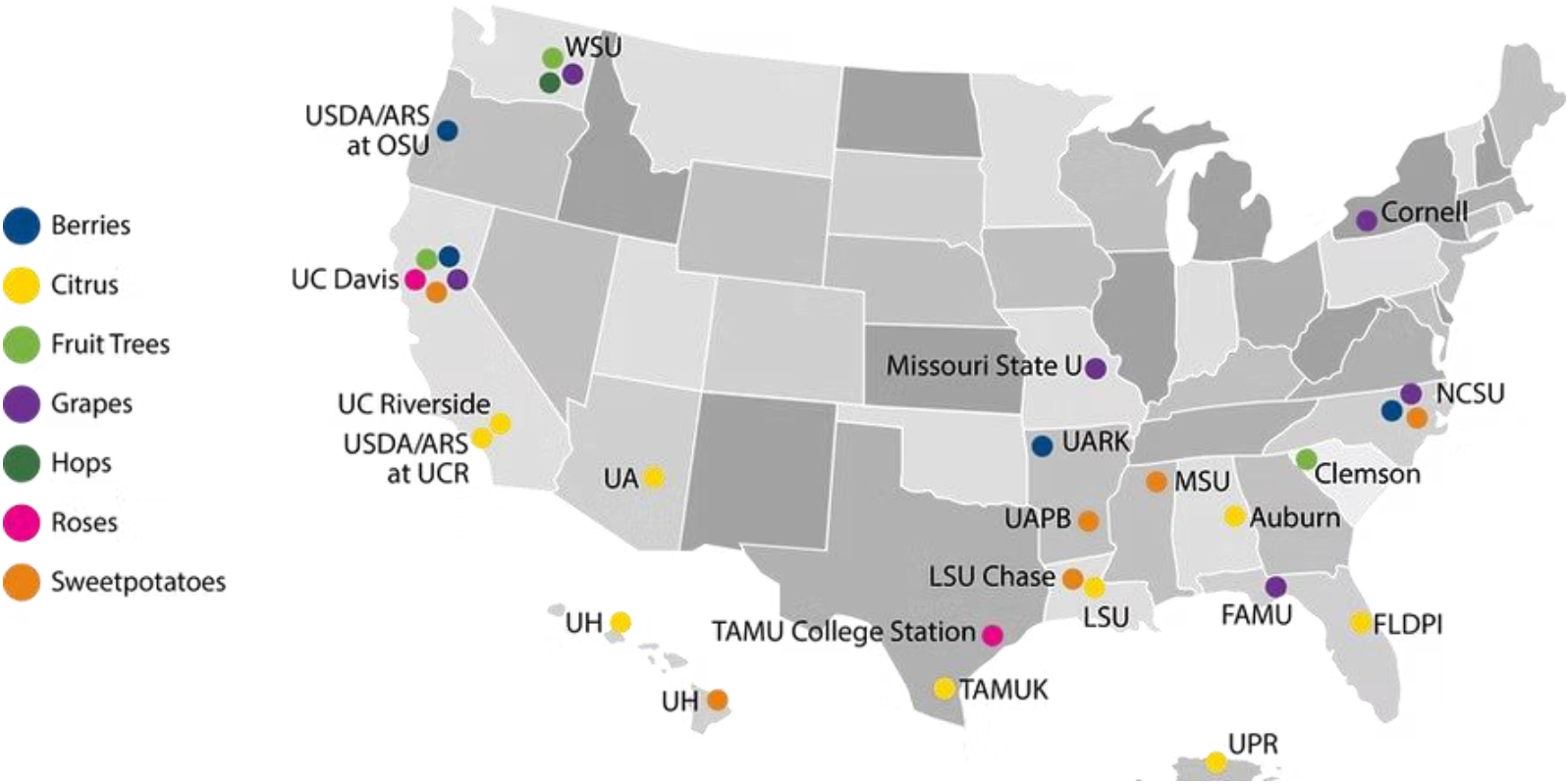


National Plant Diagnostic Network



National Clean Plant Network Centers

National Clean Plant Network Centers





State and Federal Regulatory Actors

- National Plant Board (NPB) - organization of the plant pest regulatory agencies of each State.
 - Forest Service, APHIS, Customs and Border Patrol, Ag Research Service
 - State Plant Health Directors
 - State Plant Health laboratory (often NPND labs)
- USDA Animal Plant Health Inspection Service (APHIS)
 - State Plant Regulatory Officer
 - Phytosanitary certificates for all exports
 - Plant Quarantine Labs

2020 USDA Seed Grant

- Objective: to develop consensus for a national and sustainable system for Plant Disease Validation Research.
- Meetings: over 1500 volunteer person hours
- Committees
 - Validation methods and standards
 - Laboratory test developers and researchers
 - Taxonomic experts and reference collection holders
 - Communications and IT platform planners
- Output: successful project proposal with USDA NIFA for \$1 mio to develop the Diagnostic Assay Validation Network in 2022.



Peter Bonants
U Wageningen



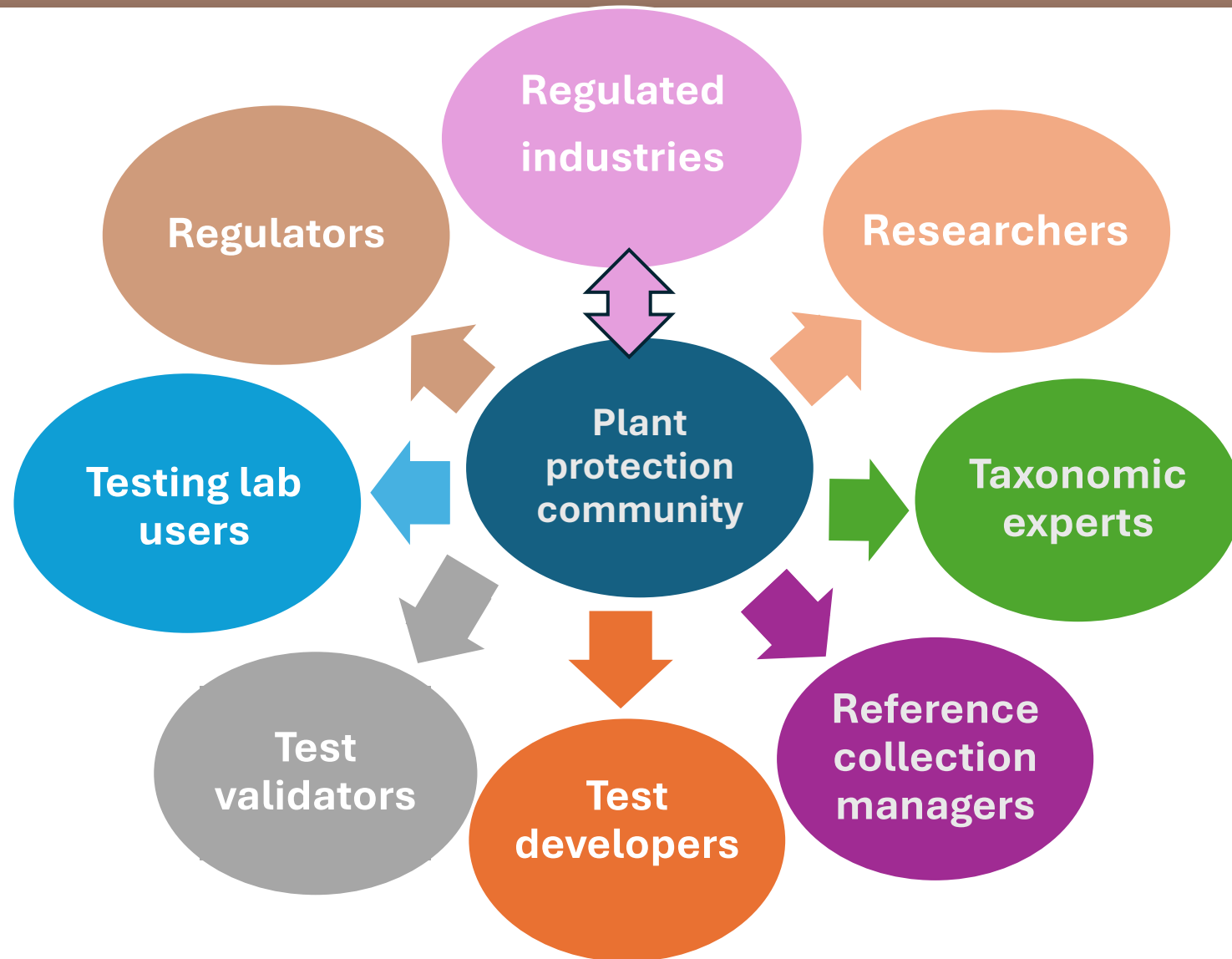
Neil Boonham
Newcastle U.



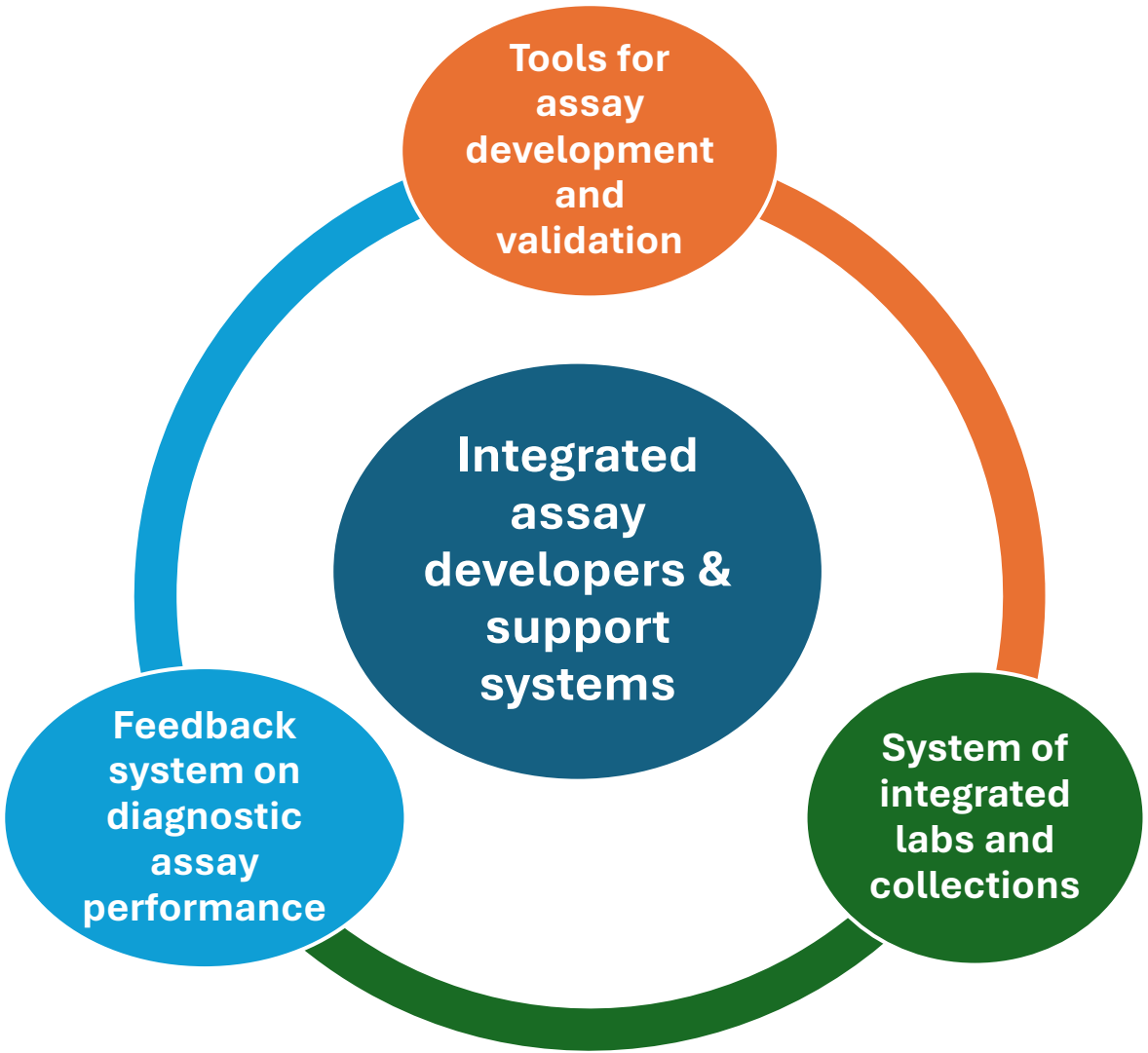
Françoise Peter
EPPO



DAVN ecosystem: producers, governments, academia



DAVN Vision



Coordinated system for diagnostic assay development and validation

Performance metric terminology and reporting standards

Resources for diagnostic assay validation

State of the Field Plant Pathogen Diagnostic Assay Development and Validation in Research Publications – 2015-2025

[10.1094/PHYTOFR-05-22-0054-FI](https://doi.org/10.1094/PHYTOFR-05-22-0054-FI)

Sharma and Luster 2023

Aim: Determine usage of validation terminology in publications about test development

Approach: Reviewed 115 peer-reviewed papers from 34 journals categorized by stated purpose and validation metrics

Key findings: only 1 study reported on 5 of 6 validation metrics

Conclusions: Few papers explicitly referenced formal validation standards; many used ad-hoc validation schemes; Core metrics were incomplete or absent

Standardizing test validation language and reporting

- Phytointiers article: Groth-Helms et al., 2023. **Terminology and Guidelines for Diagnostic Assay Development and Validation**
<https://doi.org/10.1094/PHTOFR-05-22-0059-FI>
- APS journal instructions to authors: Research manuscripts should give focus to assay validation parameters and explicitly state the purpose of the assay, assay fitness for the purpose, and which of the following validation parameters were addressed and how: **analytical specificity, diagnostic specificity, analytical sensitivity, diagnostic sensitivity, repeatability, and reproducibility.**
- Tools and terminology: Groth citation when tools are used

Phytofrontiers Special Issue 2023



Analytics

26 articles published

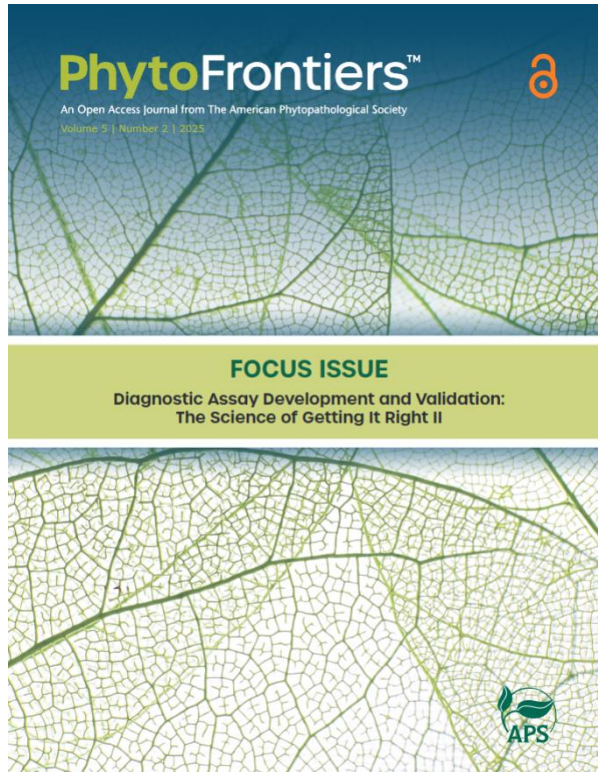
Whole issue downloads – 780 times

Articles download – 62131

Citation - 250



Phytofrontiers Special Issue 2025



How Controls Improve Diagnostic Assay Performance: Hitchhiker's Guide to Diagnostic Assay Controls

SPECIAL ISSUE - 2025
Diagnostic Assay Development and Validation: The Science of Getting It Right II

A 2023–2024 Literature Review of Validation Standards and Diagnostic Accuracy

Diagnostic Assay Validation Network (DAVN) Progress and Evolution

Analytics

17 articles published

Whole issue downloads – 226 times

Articles download – 12377

Citation - 36



Test controls – IPPC types definitions, rationale, examples

Martin, et al., 2025. How Controls Improve Diagnostic Assay Performance.

<https://doi.org/10.1094/PHYTOFR-10-24-0118-FI>

Type of controls	Endogenous internal control	Positive amplification control (PAC)	Positive extraction control (PEC)	Exogenous internal control (PAC/PEC)	Plant internal control (PIC)	Negative amplification control (NAC)	Negative extraction control (NEC)
Definition	A control that is naturally present in the sample	A sample containing the target pathogen	Sample contains target organism	A control that is spiked in the sample preparation	Same as endogenous internal control, but is plant targeted	No template control containing water	Sample containing healthy host plant DNA
Rational	To check for assay inhibition & successful extraction	To ensure the assay is working as expected	To ensure the extraction of target pathogen is successful	To monitor that the extraction, amplification, and detection work efficiently	To monitor false negative	To monitor false positive	To monitor contamination during nucleic acid extraction and/or cross-reactions with the host tissue
Example	Plant primers/probe (universal, generic, or specific) - COX and housekeeping genes	Nucleic acid from an infected plant, pure culture or synthetic DNA	Nucleic acid from artificially infected host or spiked with the target organism	Nucleic acid extract or synthetic nucleotides of known concentration and sequence	Housekeeping gene like COX or NAD	PCR grade water in the sample mix	Extraction buffer or nucleic acid extract from uninfected host tissue

Motivating test development and validation research – a second publication for authors

Research article format



Work instruction format



Volume 94, Issue 6

Research Article

Genetic characterization of Blueberry necrotic ring blotch virus, a novel RNA virus with unique genetic features

Diego F. Quito-Avila¹, Philip M. Brannen², William O. Cline³, Philip F. Harmon⁴ and Robert R. Martin⁵

 View Affiliations

Published: 01 June 2013 | <https://doi.org/10.1099/vir.0.050393-0>

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Document Control Number DAVN-WI-	WORK INSTRUCTION Diagnostic Assay Validation Network Phytofrontiers	Revision Number 1.0
Effective Date: Oct-25-2023	Blueberry necrotic ring blotch virus (BNRBV) RT-PCR	Page 1 of 3

Purpose: RT-PCR detection of Blueberry necrotic ring blotch virus (BNRBV) in symptomatic leaf tissue

WI derived from primary article: Genetic characterization of Blueberry necrotic ring blotch virus (BNRBV), a novel RNA virus with unique genetic features. 2013.

Primers:

P31-1-f: 5' CCAGTTTGGAGGAATTGCAT 3'

P31-1-r: 5' GCGTTTCAGCACCCTAAC 3'

Target amplicon size: 432 bp

Controls:

Research to Work Instruction

Research publication

- Abstract
- Introduction
 - Description of problem
 - Review of relevant literature
 - Hypothesis to be tested
- Materials and Methods
 - Isolates used
 - Gold standard comparison
 - Target/primer/probe selection
 - Sampling
 - Testing
 - Optimization
- Results
- Discussion
- Literature cited



Work Instruction

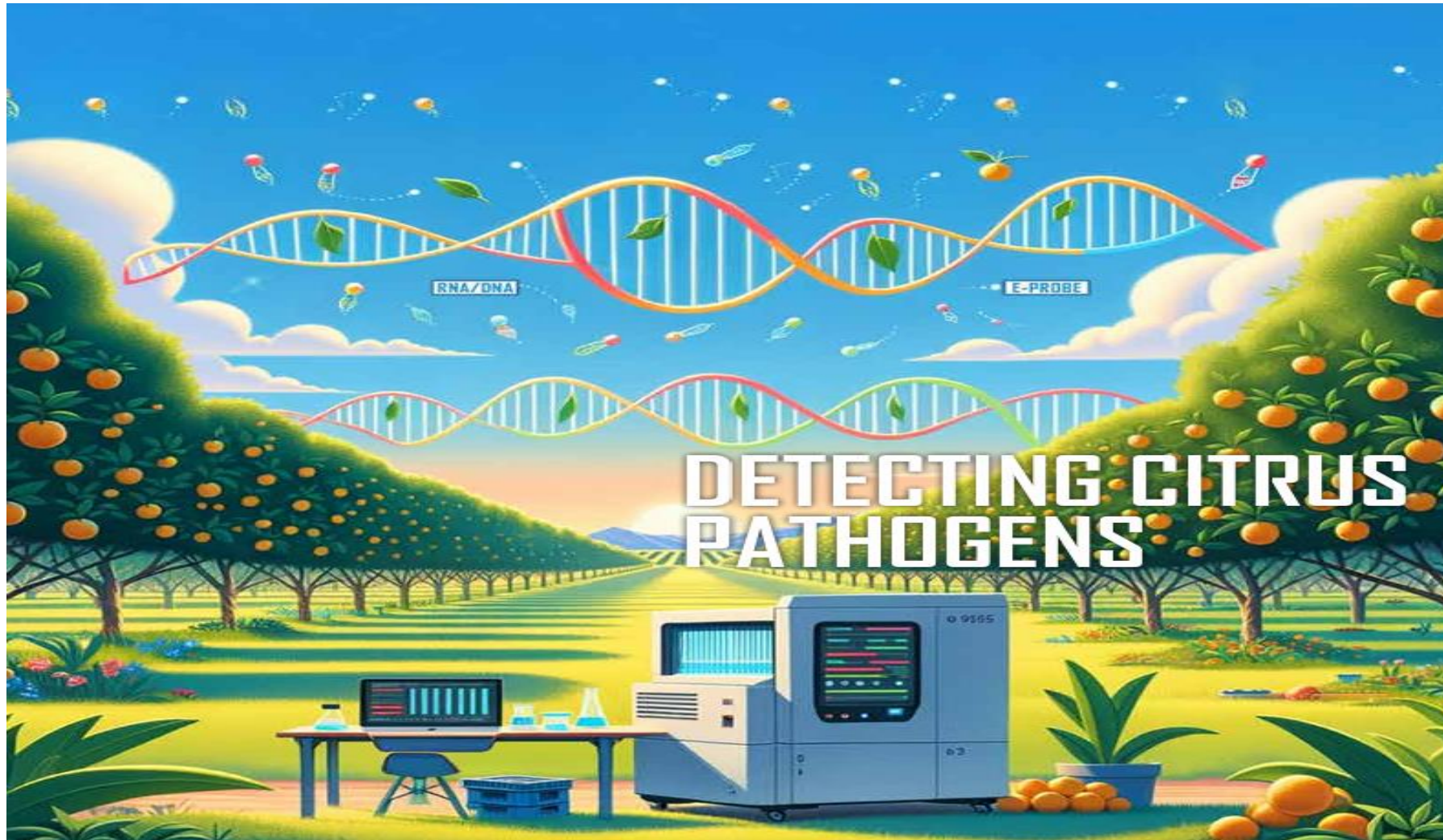
- Purpose/use of the test
- Test components
 - Primers or probes
 - Reagents & supplies
 - Controls
- Procedure
 - Sample collection
 - Sample prep
 - Reaction set-up
 - Data interpretation
- Performance summary
 - Diagnostic sensitivity
 - Specificity
 - Limit of detection
- QC and troubleshooting, Safety and storage considerations
- References



Webpage – <https://www.apsnet.org/DAVN/>
DAVN updates - <https://forms.scisoc.org/davn-info/>
DAVN mailbox - DAVN@scisoc.com



Validating HTS test data (MiFi[®] e-probes for citrus pathogens of regulatory concern)



Citrograph Magazine
Spring 2024

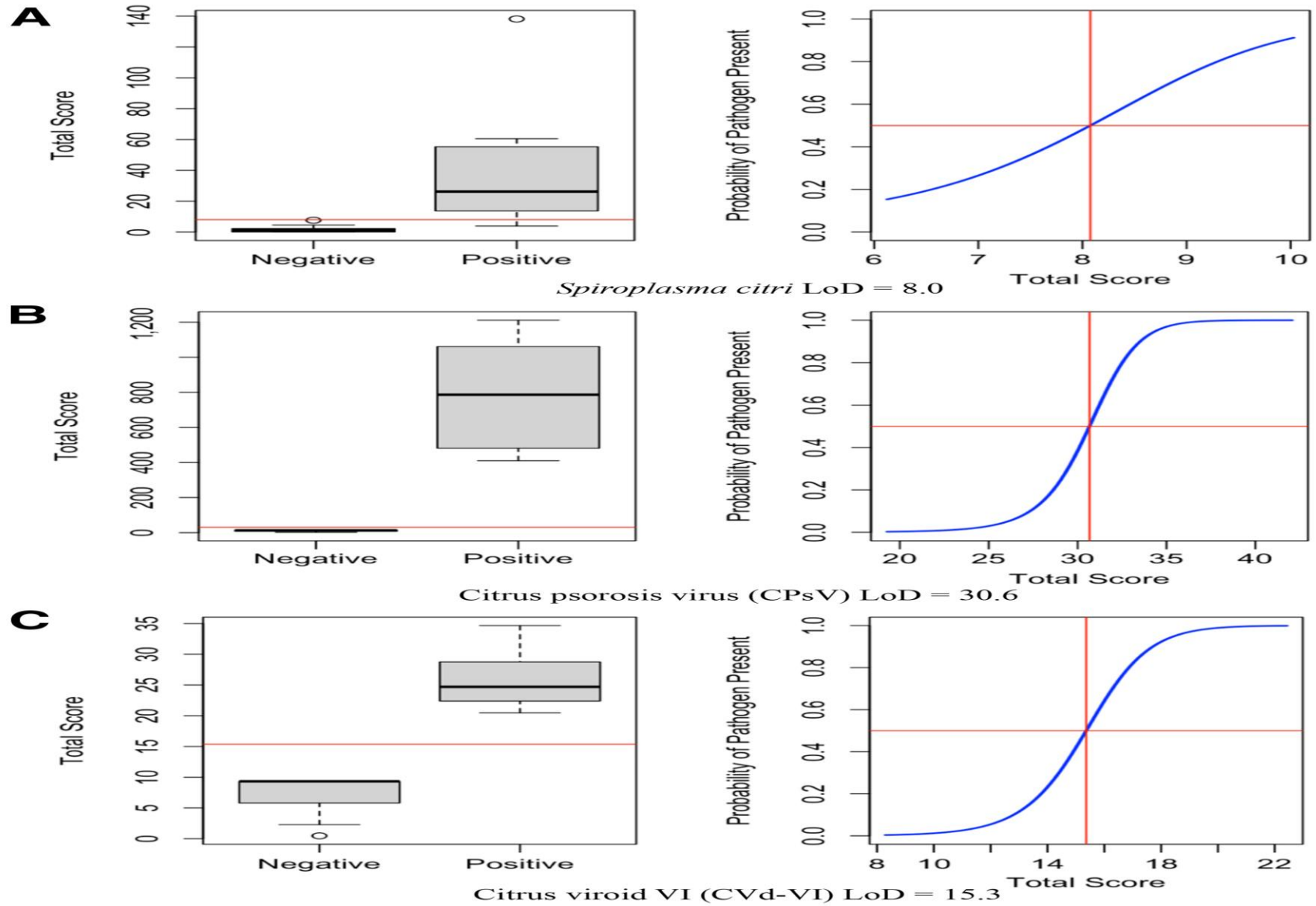
Using DAVN calculators for HTS test validation

Citrus exocortis viroid (CEVd-30-C)

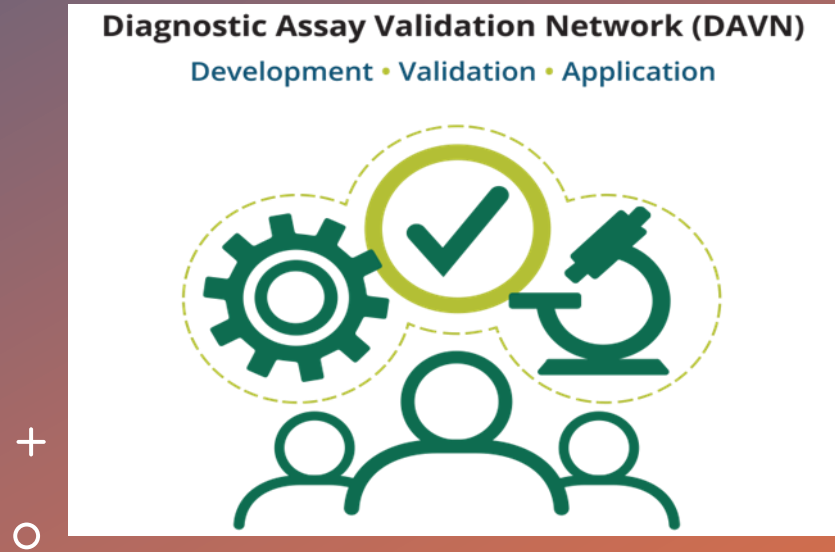
User	Sample	PCR call	p-value	MiFi call					
1	1	+	5.65E-15	positive	Repeatability within user		Precision across users		
	2	+	1.96E-01	positive	100%		100%		
	3	-	0.195683	negative					
	4	+	2.66E-11	positive					
2	1	+	5.73E-13	positive					
	2	+	0..0037	positive					
	3	-	0.195683	negative					
	4	+	2.66E-11	positive					
3	1	+	2.66E-11	positive					
	2	+	1.96E-01	positive					
	3	-	0.195683	negative					
	4	+	2.66E-11	positive					

Statistical analysis for validation of HTS test data using Electronic Diagnostic Nucleic Acid Analysis

- Nascimento, D. M., Wang, H.; Ribeiro-Junior, M. R, Campos, R., Bodaghi, S., Espindola, A.; Vidalakis, G.; and Cardwell, K.F. 2025. Development and validation of a suite of e-probes for Electronic Diagnostic Nucleic Acid Analysis (EDNA) of imported citrus budwood for 30 citrus pathogens of importance to the U.S. PhytoFrontiers V.2 <https://doi.org/10.1094/PHYTOFR-12-24-0140-FI>
- Dang, T., Wang, H., Espíndola, A. S., Habiger, J., Vidalakis, G., & **Cardwell, K.** 2022. Development and statistical validation of e-probe diagnostic nucleic acid analysis (EDNA) detection assays for the detection of citrus pathogens from raw high throughput sequencing data. PhytoFrontiersTM. <https://doi.org/10.1094/PHYTOFR-05-22-0047-FI>



Thank you to the organizers of this conference for the invitation.



And to YOU for listening!