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Plant Pathogen Confirmatory Diagnostics Laboratory

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The Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL, formerly Beltsville) develops, adapts, validates, and utilizes diagnostic methods for the detection of regulated plant pathogens.

The laboratory is committed to quality in biochemical and molecular diagnostics and is the only Federal Plant Pathology ISO/IEC 17025:2017 accredited laboratory that conducts operational diagnostics for plant pathogens of regulatory concern. The laboratory routinely tests for citrus huanglongbing (citrus greening), *Phytophthora ramorum*, *Plum pox virus* and citrus canker; molecular confirmation/identification of other regulated plant pathogens of concern is also conducted.

Laboratory programs use cutting-edge technologies from the fields of plant pathology, molecular biology, human and animal clinical diagnostics, and bio-detection to develop, adapt, and improve methods for accurate and rapid diagnosis of plant pathogens. The laboratory uses these methods to diagnose and differentiate high consequence and select agent plant pathogens that require federal confirmation. PPCDL strives to achieve timely transfer of diagnostic tools that are field deployable for PPQ emergency response and eradication programs, as well as the Cooperative Agricultural Pest Survey (CAPS) program. Tools are deployed to

stakeholders through clear-written standard operating procedures and hands-on laboratory training for end users within and outside of PPQ.

The laboratory is also a key component of the PPQ National Plant Protection Laboratory Accreditation Program (NPPLAP) and is responsible for proficiency test panel development, validation, and delivery to laboratories who perform diagnostics on behalf of PPQ. PPCDL also provides validated diagnostic controls to the National Plant Diagnostic Network (NPDN). Staff routinely conduct outreach to the plant pathology diagnostic community by providing technical support to scientists within the NPDN, PPQ port and regional identifiers, and state departments of agriculture in the detection of regulatory plant pathogens by providing protocols, hands-on laboratory training, and troubleshooting for PPQ validated diagnostics. Scientists also contribute their expertise by serving as members of scientific groups and committees.

Recent Accomplishments

Expand All

Diagnostic Tools

- Developed PCR-based assay for the differentiation of Asiatic citrus canker pathotypes.
- Developed PCR-based assay for the detection of Xylella fastidiosa subsp. *pauca* CoDiRO strain.
- Completed two Work Instructions for detection of *Phytoplasma* species: 1) General DNA Extraction from Plant Tissue Using CTAB – Qiagen DNeasy® Plant Mini kit and 2) Detection of *Phytoplasmas* by Real-time Multiplex PCR for the ABI QuantStudio 7 Flex Real-Time PCR System.
- Completed two *Ralstonia solanacearum q* PCR assays and completed a PCR multiplex assay to detect *Ralstonia solanacearum* species complex and race 3 biovar 2 strains.
- Improved *Plum pox virus* (PPV) confirmatory diagnostics by developing new work instructions for total RNA extraction and multiplex RT PCR for simultaneous detection of two genomic markers and plant internal control.

- Developed multiplex conventional PCR assay for the detection of the exotic *Peronosclerospora philippinensis* (select agent pathogen) and *P. sorghi.*
- Developed PCR-based diagnostic assays for pospiviroid seed testing.
- Developed diagnostic assays for detection of Tobamoviruses.

Operational Diagnostics

- Provided rapid PPQ confirmatory molecular diagnostics for detections of exotic high consequence quarantine and select agent plant pathogens, including pospiviroids, citrus greening, citrus canker, citrus black spot, *Phytophthora ramorum*, *Phytoplasma* sp., *Okra yellow mosaic Mexico virus* and *Cotton leaf curl Gazira virus,* potato cyst nematode, *Plum pox virus, Tomato brown rugose fruit virus, Cotton leaf roll dwarf virus, Citrus leprosis virus*, and *Ralstonia solanacearum*, among others.

Quality Management

- Ensured the continued process improvement and implementation of the Laboratory Quality Management System to meet the requirements of the ISO 17025 standard. Passed ANAB ISO 17025:2017 external audit and maintained accreditation status.

High Throughput Sequencing Technologies

- Conducted diagnostics of viral pathogens using high throughput sequencing (HTS) technologies and bioinformatics analysis. Completed high throughput sequencing of several plant viruses of importance using Illumina and MinION technologies.

Outreach/Training

- Strengthens PPQ’s domestic pest preparedness, surveillance, detection, management, and eradication programs by providing hands-on laboratory training to diagnosticians of the National Plant Diagnostic Network, State Departments of Agriculture, universities, and PPQ regional identifiers.

Contact

Deshui Zhang

Laboratory Director

Email: deshui.zhang@usda.gov

Phone: 301- 313-9200

MAILING ADDRESS

BARC-East, Bldg. 580

Powder Mill Rd., Laurel, MD

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