



**CALIFORNIA STATE SCIENCE FAIR
2015 PROJECT SUMMARY**

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Project Title A Rapid Field Detection of Liberibacter Bacteria Using Lateral Flow Technology	
Abstract Objectives/Goals Citrus huanglongbing (HLB), a bacterial disease spread by the Asian citrus psyllid, has killed 50% of Florida citrus within ten years of introduction. Delayed detection and heavy pesticide use have resulted in serious epidemics and environmental pollution. The goal of this project was to develop an affordable, point of need testing to facilitate instant management decisions for growers and to complement regulatory efforts by helping them focus in high risk areas. Using a tomato model system, and an inexpensive reaction setup, a 30 min dipstick assay was developed using isothermal amplification and lateral flow technology. The results using the home detection system was comparable with lab-based assays. Since new species of Liberibacter have caused problems in laboratory diagnosis leading to epidemics in other countries in the past, a universal real time PCR detection system was developed using bioinformatic analysis of multiple bacterial genomes. Overall goal is to promote rapid early detection to facilitate better disease management and protect the citrus industry in California. Methods/Materials A \$20 prototype for isothermal amplification, Bst DNA polymerase, primers, primers labeled with biotin and FAM, dipstick assay components (provided by GE Healthcare), anti-FITC antibody conjugated to gold, streptavidin, anti-rabbit antibody, real time PCR machine, healthy and infected tomato, psyllid and bacterial DNAs. Results An isothermal amplification was developed in the lab and adapted for field detection using a \$20 prototype. A dipstick assay was developed capable of distinguishing healthy and infected plants and insects. Several primers developed in this study detected four species of Liberibacters, but not other closely related bacteria, in qPCR assays. Conclusions/Discussion A simple to use and affordable home detection system based on dipstick assay was developed in this study to help both growers and regulators to avoid delays in the current system of lab-based centralized detection system. It is expected to greatly help growers and home owners to manage their citrus better and for judicial use of pesticide. In 2004, Brazil failed to contain HLB because of emergence of a new species of bacterium. Using bioinformatics analysis of multiple Liberibacters and other closely related bacteria, universal primers capable of selective detection of multiple Liberibacter species was developed here for qPCR.	
Summary Statement A simple and affordable dipstick assay was developed for instant detection of plant pathogens to help growers protect citrus from the deadly huanglongbing disease and to encourage judicial use of pesticides.	
Help Received Laboratory facilities provided by Dr. Richard Lee. I appreciate the technical guidance I received from the lab members.	