Developing CANARY® Assays for Plant Pathogen Detection

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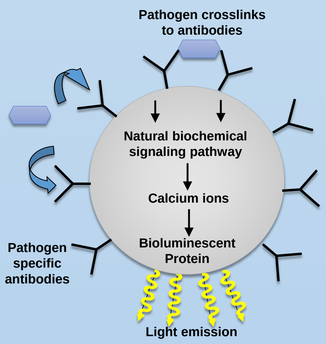
Introduction

CANARY® (Cellular Analysis and Notification of Antigen Risks and Yields) is an immunological assay that utilizes engineered biosensors expressing target specific antibodies on their surface. Binding to target triggers activation of the CANARY® cells, causing them to luminesce. Multiple instrument platforms and proprietary software algorithms measure and interpret this bioluminescence response. Due to innate characteristics of the CANARY® biosensors the CANARY® technology is able to combine the sensitivity of PCR with the speed of lateral flow devices. A CANARY® biosensor can be made, and assays developed, to detect any target for which an antibody can be created, including bacteria, viruses, fungi, oomycetes, and toxins. CANARY® assays have been developed for *Ralstonia solanacearum* and are currently in development for *Citrus leprosis viruses* cytoplasmic type (CiLV-C1 and CiLV-C2) and *Phytophthora* spp. The *Ralstonia* assay has been field-tested with geranium cuttings at PPQ Plant Inspection Stations and demonstrated no erroneous results; the assay can be completed within 5 minutes and received positive feedback for its ease of use. The *Citrus leprosis* assay requires little over 5 minutes to complete after sample excision. It has been tested with infected sweet orange leaves obtained from Mexico and has demonstrated >98% positive and negative predictive values for CiLV-C1, using RT-qPCR as gold standard. It did not show cross-reactivity with CiLV-C2 or *Citrus leprosis virus* nuclear type (CiLV-N). The *Phytophthora* assay is in early development, but has been used to successfully detect all 11 species of *Phytophthora* tested, with no cross-reactivity to *Pythium*. These data demonstrate that the CANARY® technology can be an attractive platform for the accurate and rapid screening for plant pathogens in an easy-to-use format.

Comparison of CANARY® with Other Immunoassays

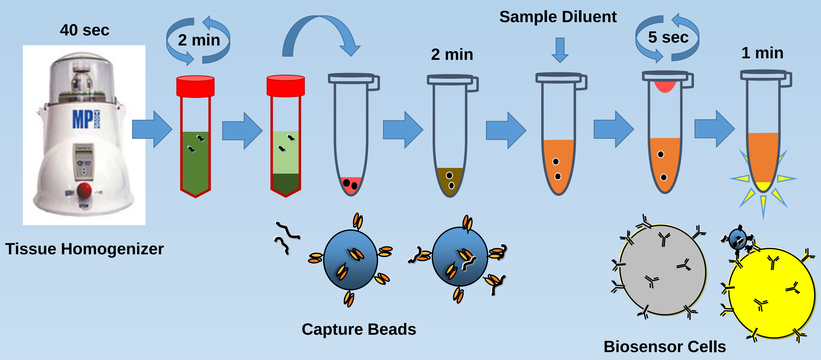
	Speed	Sensitivity	Ease of use	Multiplexing capability
ELISA	-	+	-	+
Lateral Flow	+	-	+	-
CANARY®	+	+	+	+

CANARY® Assay Principle

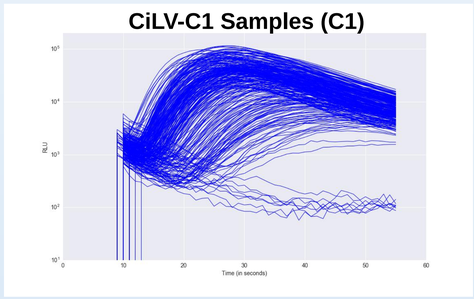


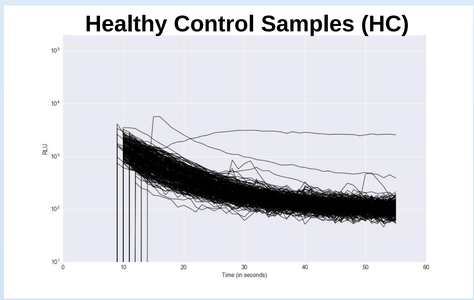
Citrus Leprosis Symptoms

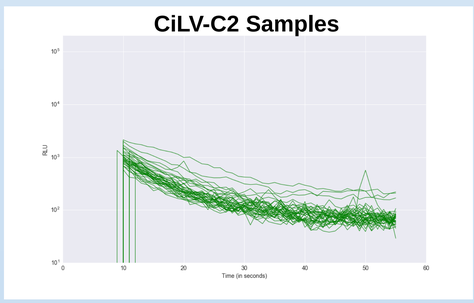
CiLV-C1 Assay Protocol

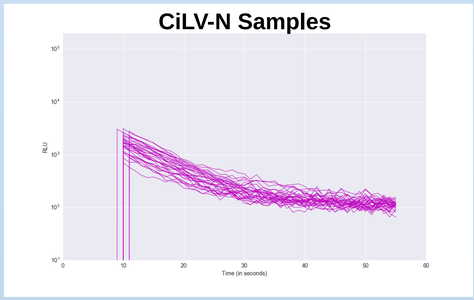


CiLV-C1 Assay Raw Data









CiLV-C1 Assay Statistics

- Healthy control sweet orange leaf samples (**HC**): 109
- CiLV-C1 samples (**C1**): 101
- Sensitivity: **98.1%** (CI = 93% to 99%)
- Specificity: **99.1%** (CI = 95% to 99%)
- Positive predictive value: **99.0%** (CI = 94% to 99%)
- Negative predictive value: **98.3%** (CI = 94% to 99%)
- No cross-reactivity (100% negative algorithm calls) for CiLV-C2
- No cross-reactivity (100% negative algorithm calls) for CiLV-N

Conclusions

The CANARY® assay has been developed and validated as a screening technique for the detection of CiLV-C1 in sweet orange leaves using field samples collected from an area in which CiLV-C1 is endemic.

Future Work

1. Test CiLV-C1 infected mites.
2. Develop a CANARY® assay for the detection of CiLV-C2.

Acknowledgement

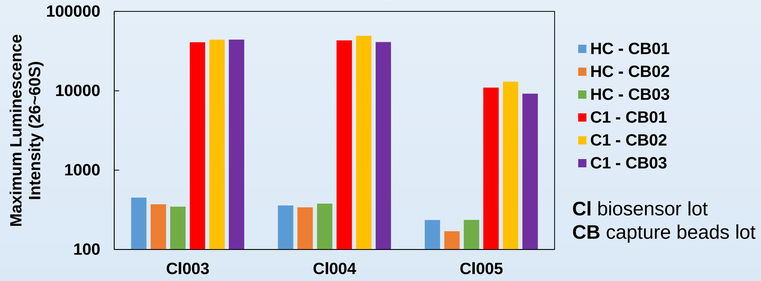
We thank Dr. Gabriel Colina for the CiLV-C1 field samples.

References

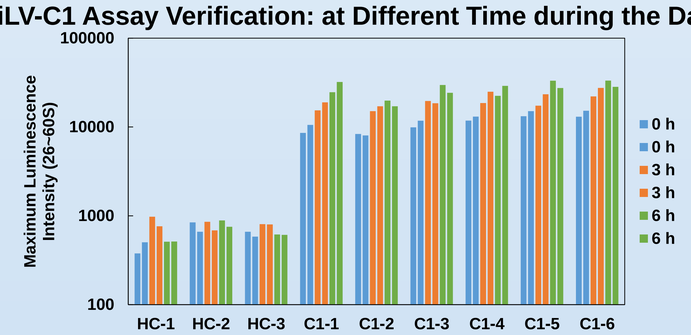
1. Rider, Petrovick, Nargi, Harper, Schwoebel, Mathews, Blanchard, Bortolin, Young, Chen, Hollis. A B cell-based sensor for rapid identification of pathogens. Science. 2003 Jul 11;301(5630):213-5.
2. CPHST Laboratory Beltsville NPGBL 2009 Annual Report.

CiLV-C1 Assay Verification Testing

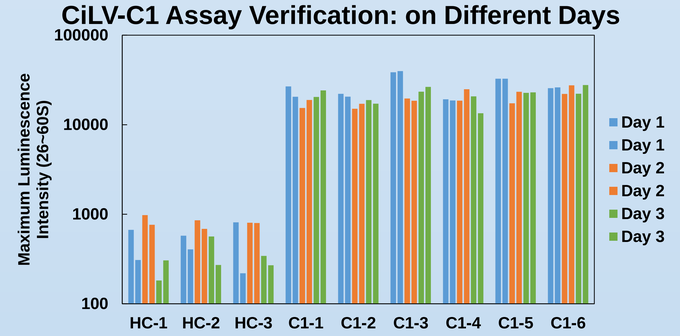
CiLV-C1 Assay Verification: with Different Lots of Biosensors and Capture Beads



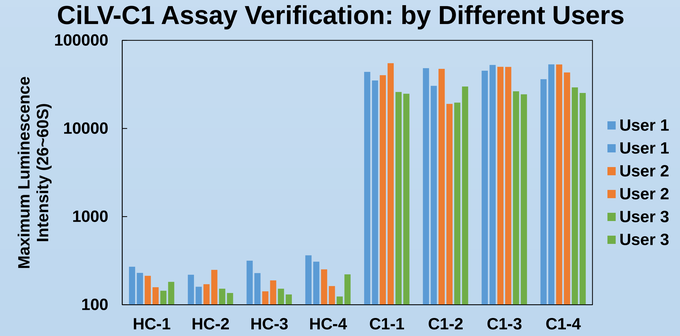
CiLV-C1 Assay Verification: at Different Time during the Day



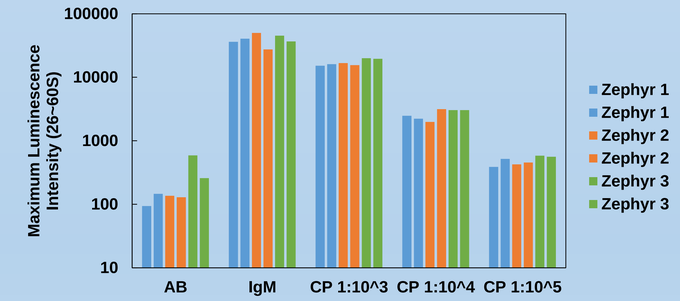
CiLV-C1 Assay Verification: on Different Days



CiLV-C1 Assay Verification: by Different Users



CiLV-C1 Assay Verification: with Different Instruments



*** CP** (recombinant CiLV-C coat protein modified beads) was used as positive controls.

Phytophthora Assay

- *Phytophthora* assay is currently under development
- Has been shown to detect all *Phytophthora* species tested with no cross-reactivity to *Pythium*
- Scheduled for completion in mid-2016
- Looking for collaborators to supply and/or test field samples