



Photo Source: Clemson University - USDA Cooperative Extension Slide Series, Bugwood.org

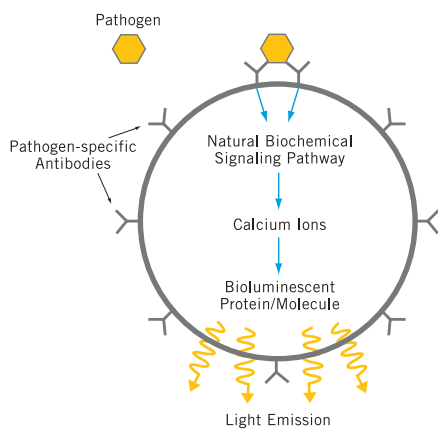
Ralstonia Zephyr Assay

The CANARY®- Zephyr System allows for unparalleled detection of low levels of *Ralstonia solanacearum* in 3 minutes.

CANARY® technology was developed by MIT-Lincoln Laboratory under a DARPA contract, and commercialized by PathSensors, Inc. The technology utilizes biosensors created from B lymphocytes (white blood cells) modified to express surface-bound, target-specific antibodies and a bioluminescent protein. When the biosensor binds to its target pathogen, the antibodies trigger the intracellular release of calcium. This calcium causes the bioluminescent protein to emit light. Sophisticated algorithms analyze this light output, resulting in definitive “positive” or “negative” test results.

Advantages of this system are its extreme speed and sensitivity. The speed of detection is a result of rapid intracellular signaling. The sensitivity is achieved through signal amplification within the cell. This leading edge technology identifies targets in 3 minutes with analytical sensitivities down to 10's of CFU of target per sample.

CANARY® Biosensor



Assay Specifications

Analytical Sensitivity (LoD)	74 cfu <i>Ralstonia solanacearum</i> (all races and biovars)
Assay Sensitivity	100 cfu
Resistance to Interferents	0 false positives with 14 closely related bacterial taxa
Time to Results	3 minutes

Note: Performance validated by USDA APHIS PPQ, Beltsville, MD and FERA, London, UK



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Testing Protocol:

Add plant samples to Assay Buffer and shake vigorously for 5-10 seconds



Transfer 200 µL to Assay Tube



Start Zephyr program, enter operator ID, scan biosensor ID



Centrifuge sample for 2 minutes



Add biosensors



Centrifuge 5 seconds



Transfer sample to luminometer (read for 1 minute)



RETRIEVE RESULTS

Testing and Results:

The **CANARY®-Zephyr *Ralstonia*** assay has been extensively tested for the ability to detect *Ralstonia solanacearum*, both alone and in combination with a variety of sample matrices.

The *Ralstonia* assay was validated with:

- 60 *Ralstonia solanacearum* isolates
- 14 potential cross-reacting bacterial taxa
- 3 different greenhouse-grown plants infected with *Ralstonia* isolates
- 30 river water samples
- 24 potato tuber samples

All 60 *Ralstonia* isolates were positively identified in the absence of plant material as well as in greenhouse-grown plants and potato tuber samples. All 14 bacterial taxa generated negative results, as did all 30 river water samples.

Technologies for the detection of *Ralstonia solanacearum*

Technology	Approx. Time	LoD (cfu)
PathSensors: CANARY®-Zephyr	3 minutes	<100
qPCR	30-96 minutes	100
ELISA	3-24 hours	10 ⁴ - 10 ⁶
Lateral flow	3-30 minutes	10 ⁴ - 10 ⁷

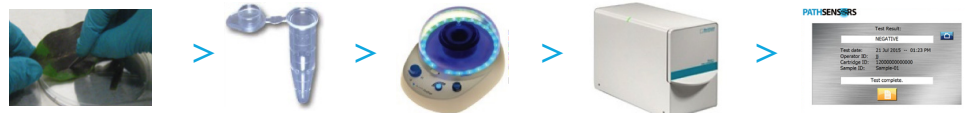
Table generated using information found in New Pest Response Guidelines: *Ralstonia solanacearum* race 3 biovar 2; P Berger et al, USDA-APHIS-PPQ. http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg-ralstonia.pdf



CANARY® technology delivers the sensitivity of qPCR at the speed of immunoassays.

PATHSENSORS

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