



Rapid Detection of Citrus Black Spot with Loop-Mediated Isothermal Amplification (LAMP)

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United States Department of Agriculture



Citrus Black Spot (CBS)

(Phyllosticta citricarpa, Guignardia citricarpa)

Disease Symptoms



Causes unsightly lesions on fruit rind.

Confirmation

March of 2010. Orange grove in Collier County, FL.
CBS is regulated in three FL Counties.



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Fresh Fruit Exports



Regulation at Packing Facilities

Citrus Health Response Program

Season: September to April for EU shipments

Hours: 5:00am- 9:00pm including weekends and holidays

PPQ identifiers remain on-call beyond normal work hours



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Phyllosticta citricarpa Detection by LAMP

Symptomatic Tissue



Cut out lesion



Crush with pestle and let soak in buffer 1 min



Spin 1400rpm 30s



20µl on LiNK column



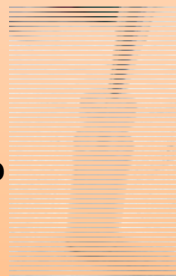
Let sit 5 minutes



Compress column



2µl for LAMP

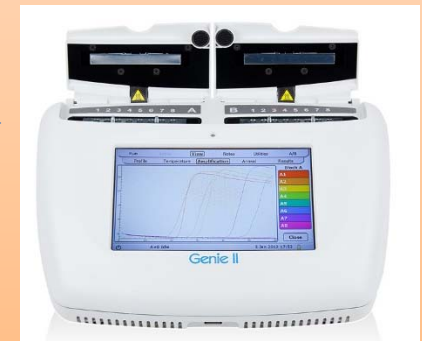


25 µl reaction



12.5 µl Isothermal Master Mix
+
9.5 µl H₂O
+
1.0 µl LAMP Primer Mix

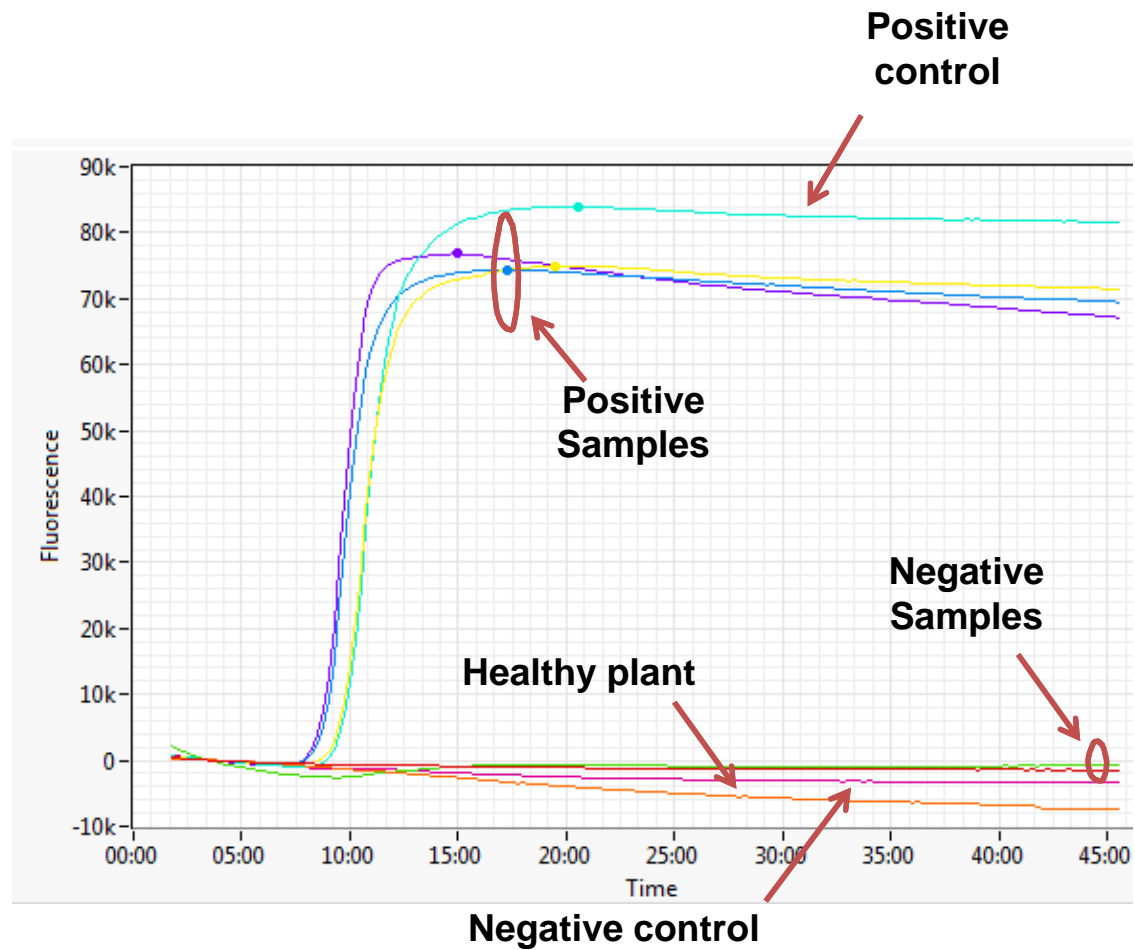
Genie II LAMP



Sample preparation 12 minutes / LAMP amplifications 8-20 minutes



Phyllosticta citricarpa Detection by LAMP





Citrus Black Spot

Next Harvest Season

PPQ officers at packing houses can use this technology system to quickly back-up their determinations on whether a shipment can be shipped or must be held for lab testing.

Conclusions

- LAMP offers a fast and accurate way to detect *P. citricarpa*
- LiNK columns provide a faster extraction method, detectable by LAMP and Real-Time PCR
- LAMP has been able to reproduce Real-Time PCR results using environmental samples (55/55 matched results)



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