# VALIDATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS FOR FIELD DETECTION OF RALSTONIA SOLANACEARUM SPECIES COMPLEX STRAINS

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## Introduction

- Seed potato certification have zero tolerance for bacterial wilt disease caused by *Ralstonia solanacearum* species complex (RSSC) strains.
- Current seed potato certification process entails use of time and resource consuming laboratory based diagnostic techniques such as ELISA and PCR.
- Loop-mediated Isothermal Amplification (LAMP) assays for detection of potato brown rot strain (sequevar 1)[1] (LAMP I) and RSSC strains (LAMP II)[2] have recently been developed.
- We tested the field applicability of real-time fluorescent LAMP assays[1,2] were tested along with alkaline PEG extraction method as an alternative to ELISA test for the detection of RSSC strains under both laboratory and field conditions using Genie II and BioRanger (Fig 1).

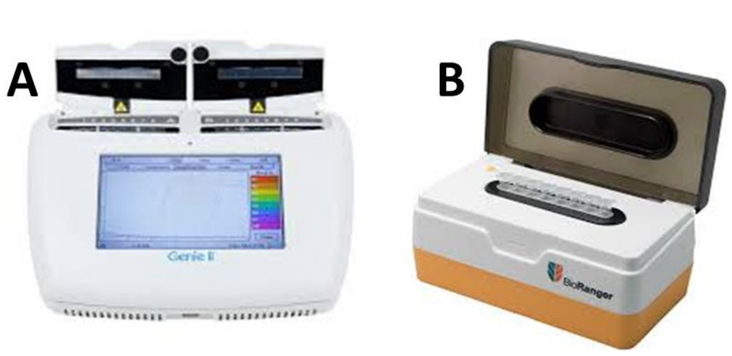


Figure 1. Field deployable LAMP instruments, A) Genie II (Optigene, UK) and B) BioRanger (Diagenetix, USA)

## Material and methods

**Lab based comparative evaluation of LAMP assay and ELISA test**:

- Bacterial strains: 21 RSSC wide strains, 1 Dickeya, 3 Pectobacterium, and 1 Salmonela strains tested for assay specificity
- Pure culture of sequevar 1 strains was used in dilution series experiment for assay sensitivity
- DNA extracted from potato stem and tuber samples using alkaline PEG buffer[3]
- Soil samples first suspended in phosphate buffer (pH 7) followed by DNA extraction by alkaline PEG buffer. In addition purified total DNA extracted from soil using PowerSoil Kit (MoBio).
- Isothermal master-mix (ISO-dr001, dr004), and 5 primer set (FIP, BIP, F3, B3, BL) for LAMP I[1] and six primer set (FIP, BIP, F3, B3, FL, BL) for LAMP II[2] were used
- KEPHIS' customer soil samples were first sieved and homogenized. Homogenized samples were divided into 2 portions, half used for the routine post-enrichment DAS-ELISA test by KEPHIS staff and the other half for direct LAMP assays.

**Field validation of LAMP assay**: Soil, potato stem, tuber samples

- Soil sample: about 5 g composite soil suspended in 45 ml phosphate buffer, 10 µl supernatant diluted in 90 µl alkaline PEG solution, 3 µl lysate used directly as template in LAMP II assay.
- Stem sample: 0.5 cm length stem base from 5 to 10 symptomatic potato plants per field gently macerated in 2 ml Alkaline PEG buffer in plastic maceration bags, , 3 µl supernatant directly as a template for LAMP reaction.
- Potato tuber samples: macerated cored tuber samples from 50 ml Falcon tube attached to the coring device were diluted in alkaline PEG solution (1:10) in 1.5 ml Eppendorf tubes, 3 µl supernatant directly as a template for LAMP reaction.

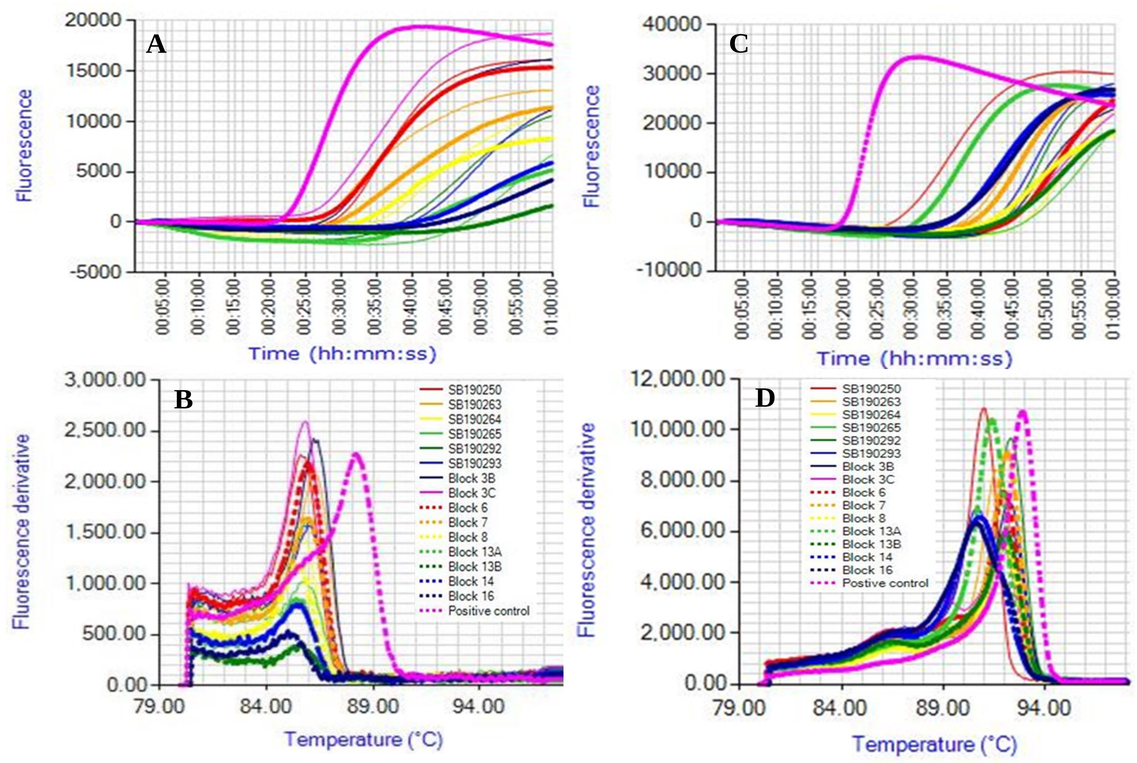


Figure 2. A) Amplification curve, soil samples lysed in Alkaline PEG200 solution; B) Anneal (melting) temperature, soil samples lysed in Alkaline PEG200; C) Amplification curve, soil samples, purified DNA; D) Anneal (melting) temperature, soil samples, purified DNA

## Results and discussion

- LAMP I was specific to sequevar 1 and 2 strains, LAMP II detected all phylotypes I, II, and III strains tested.
- Both LAMP I and II assays detected up to $10^4$ CFU per ml bacteria from stem and tubers samples under 30 minutes.
- The detection limit from soil samples was as low as 100 CFU per gram of soil.
- In comparative test with standard DAS-ELISA tests, LAMP II detected 18 out of 29 soil samples (time to positivity ranging 43-58 minuets) that tested negative with ELISA.
- Direct soil LAMP with alkaline PEG extraction was comparable to LAMP with purified DNA (Fig 2).

**Table 1. Comparison of LAMP assay over alternative tests for BW disease diagnostic tools, ELISA, PCR, and qPCR**

| Diagnostic Tools | On-site Testing[1] | Time to Get Results (hr)[2] | Cost/100 Samples ($)[3] | Strain Specificity[4] |
|---|---|---|---|---|
| LAMP | Yes | 0.5–0.8 | 300 | Very high |
| ELISA | No | 96-120 | 350 | Low |
| PCR | No | 5 | 700 | High |
| qPCR | No | 2 | 1,000 | Very high |

[1]Testing on on-farm potato fields or at point of entry (quarantine control).

[2]From receiving samples to getting test results for decision-making. Current turnaround time for ELISA is at least 1 week.

[3]Excluding labour costs, which are significantly higher than LAMP.

[4]Detection of the entire RSSC strains or specific to potato brown rot strains.

## Conclusion

- The LAMP assays were robust even with crude potato plant tissue and soil samples with better performance than the standard ELISA tests.
- The LAMP assays offers phytosanitary regulators the convenience of on-site testing of RSSC strains from soil, stem and tuber samples without the need for complicated sample processing needed in standard ELISA and PCR procedures (Table 1).

## References

- [1]Kubota and Jenkins, 2015. https://doi.org/10.3390/ijms16034786
- [2]Lenarcic et al., 2014. doi: 0.1371/journal.pone.0096027
- [3]Chomczynski and Rymaszewski, 2006. DOI: 10.2144/000112149

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