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An on-site adaptable test for rapid and sensitive detection of Potato mop-top virus, a soil-borne virus of potato (Solanum tuberosum)

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ABSTRACT

Potato mop-top virus(PMTV) is considered an emerging threat to potato production in the United States. PMTV is transmitted by a soil-borne protist, *Spongospora subterranean*. Rapid, accurate, and sensitive detection of PMTV in leaves and tubers is an essential component in PMTV management program. A rapid test that can be adapted to in-field, on-site testing with minimal sample manipulation could help in ensuring the sanitary status of the produce in situations such as certification programs and shipping point inspections. Toward that goal, a rapid and highly sensitive recombinase polymerase amplification (RPA)-based test was developed for PMTV detection in potato tubers. The test combines the convenience of RPA assay with a simple sample extraction procedure, making it amenable to rapid on-site diagnosis of PMTV. Furthermore, the assay was duplexed with a plant internal control to monitor sample extraction and RPA reaction performance. The method described could detect as little as 10 fg of PMTV RNA transcript in various potato tissues, the diagnostic limit of detection (LOQ) similar to that of traditional molecular methods.

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1 Cut core tubers with blade. Take 3 - 4 cores per tuber.

2 Extract with GEB extraction buffer at a ratio of 1:2 (w:v) in a mesh extraction bag (0.3 g/3 mL). Let rest for 5 minutes at room temperature.

3 Remove one colored PD1 filled tube for each sample being tested. Individual tubes may be cut from the strip of tubes using scissors.

4 Transfer 5 µL of sample extract into the tube containing PD1 diluent and mix well.

5 Press the “Execute Reaction” button on the AmpliFire®. Then Scan PMTV Product Code.

6 Remove a canister of reaction pellets from the white foil pouch labeled with the barcode. Then remove a strip of reaction pellets from the desiccated container. Note: Reaction Pellets are light sensitive. Immediately place remaining reaction pellets back into the desiccated tube and then insert the desiccant tube into the foil pouch to protect from light.

7 Transfer 25 µL from the colored tube from step 4, into the reaction pellet (clear tube). Mix well and spin down.

8 Press “Start” on the AmpliFire. Immediately follow the prompts to add your reactions, press “OK”, and put the lid down.

9 After 4 minutes of incubation remove the reaction(s) from the AmpliFire. Quickly mix, spin, and reinsert the reaction(s) into the AmpliFire to continue monitoring results. Take care to ensure the tubes are in their original positions and orientations.

10 The test lasts 20 minutes and the results will be visible on the screen, and should be interpreted as follows: Blue curve = FAM = PMTV. Red curve = CalRed = Internal control. (+) = Positive for PMTV (-) = PMTV not detected (!) = Invalid

11 Note: Adapted and modified from the manual of Agdia AmplifyRP® XRT for PMTV Rapid RNA Amplification Test Kit, Product No. XCS 12501

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