

# MULTIPLEX DETECTION OF VIRAL AND BACTERIAL PATHOGENS WITH THE LUMINEX TECHNOLOGY, A FLUID MICROARRAY SYSTEM

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Pathogenic viruses and bacteria pose a constant threat to the production of plant propagation material. For implementing effective disease management strategies, reliable and suitable methods for high throughput screening of the multiple pathogens are indispensable. Here we describe an immunoassay, based on the Luminex xMAP® technology, which enables detection of different plant pathogens simultaneously (so called multiplex detection). The assay requires minimal sample preparation, is performed in 96 wells microtiterplates and can be completed within 60 min. No washings are required. With the high throughput screening system of Luminex over 10.000 samples can be analyzed daily. The panel of assays can be adapted by adding a new bead set to an existing bead mixture. The sensitivity is largely similar to that of ELISA.

The Luminex technology is based on the use of microspheres (beads), internally stained with two fluorochromes. An assortment of 100 bead sets is available, each with a unique composition of red and infrared fluorochromes, which allows detection of up to 100 different pathogens. Both antibodies and nucleic probes can be covalently linked to the activated microspheres. In a microsphere immunoassay (MIA), plant extracts are subsequently incubated for 20 min with the antibody coated beads and for 20 min with secondary antibodies, conjugated with Alexa532, a reporter dye. Within the Luminex analyzer, one laser excites the internal dyes that identify each microsphere particle, and another laser the reporter dye on the secondary antibodies captured.

We developed a MIA for detection of potato virus X (PVX), potato virus Y (PVY) and potato leafroll virus (PLRV) in leaf extracts. A second MIA was developed for detection in tuber peel extracts of the bacterial pathogens *Clavibacter michiganensis* subsp. *sepedonicus* (Cms), *Erwinia carotovora* subsp. *atroseptica*, *Erwinia chrysanthemi* and *Ralstonia solanacearum* (Rsol) causal organisms of respectively ring rot, blackleg, stem rot and brown rot.

We were able to detect simultaneously Cms, Rsol, PLRV, PVX and PVY in a potato peel extract. Rsol and Cms could be detected in a multiplex setting at a detection level of ca.  $10^5$  cfu per ml. The MIA was compared with ELISA for detection of PVX in naturally infected leaf extracts. From 96 samples, 39 were positive and 56 were negative in both tests. One sample was negative in MIA and positive in ELISA.

These results indicate that MIA can be reliably used for (multiplex) detection of viral and bacterial pathogens in crude plant extracts. The technology is an attractive and cost effective alternative for other detection methods, including ELISA. The hand on time is limited, because no washings are done and only low amounts of immunoreagents are required.