

Srihunsu Malichan 2008: Development of Rapid Test Kit for Detection of Cucumber Mosaic Virus by Immunochromatographic Technique. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Ratchanee Hongprayoon, Ph.D. 83 pages.

A rapid immunochromatographic assay (immunostrip) was developed for the detection of *Cucumber mosaic virus* (CMV). The system employed three antibodies including anti-CMV monoclonal and polyclonal antibodies (MAb-CMV, PAb-CMV) and rabbit anti-mouse polyclonal antibody (RAM). MAb-CMV was conjugated with colloidal gold particles for antigen capture at the conjugate release pad (CRP). The antigen-antibody complex was capillary transported onto the Prima 40 nitrocellulose membrane where PAb-CMV and RAM were immobilized at the concentration of 1 mg/ml on test line and control line, respectively. A 33 glass fiber and 470 cotton linter were used as sample absorption pad (SAP) and wicking pad (WP) according to the manufacturer. The strip size was 0.5 x 6 cm². The efficiency test of the developed immunostrip was assessed by testing with CMV-contaminated tobacco sap. The sensitivity at 0.048 µg/ml showed clear test line and no cross reaction to healthy sap or extraction buffer was observed. This assay can be completed in 6-15 min and the intensity of the reaction was proportional to virus concentration in the sample.

The possibility to replace gold particles with dye-entrapped liposome as a detection reagent in the strip was studied. The liposome was prepared from lecithin, cholesterol and phosphatidylethanolamine in a molar ratio of 8: 10: 1. Sulforhodamine B (SRB) fluorescence dye was entrapped. Transmission electron micrograph showed an average size at 256 nanometer in diameter of the SRB-entrapped liposome. The reaction of immunoliposome was determined by Dot immunobinding assay (DIBA) with purified CMV, CMV coat protein and rabbit anti-mouse IgG.

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Thesis Advisor's signature

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