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Clark M F & Adams A N. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-83, 1977.
[East Malling Research Station, Maidstone, Kent, England]

A new serodiagnostic method for plant viruses, enzyme-linked immunosorbent assay (ELISA), was described. Morphologically different plant viruses were detected in purified preparations and in unclarified extracts of infected plants. Virus concentration in the samples was proportional to the colour intensity of the enzyme-hydrolysed substrate. [The *SCI*® indicates that this paper has been cited in over 925 publications, making it the most-cited paper from this journal.]

ELISA and the Plant Virus

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In 1972 I took up a research position at the prestigious East Malling Fruit Research Station. Part of my brief was to devise improved detection methods for the viruses of fruit and hops. Low concentrations and erratic distribution of the viruses in fruit trees, as well as an abundance of virus inhibitors and inactivators in leaf extracts, restricted indexing procedures to laborious graft transmission tests to indicator varieties or to frequently unreliable inoculations to herbaceous test plants. About this time the sporadic occurrence of the aphid-borne plum pox (sharka) virus in nurseries and orchards was causing concern. Dr. Tony Adams was given the job of monitoring the disease and spent many frustrating hours trying to develop a satisfactory radial diffusion serology test to detect the presence of this filamentous virus.

Then, at the Virology Congress in Madrid in 1975, I noticed a poster describing the microplate method of ELISA for detecting rubella virus by Dr. Alister Voller and colleagues from the Nuffield Institute of Comparative Medicine, London. Alister was intrigued by the possibility of applying the method to plant pathogens and readily agreed to collaborate in some trials with our plant viruses. Plum pox virus was the obvious first choice while arabis mosaic virus, a pathogen of strawberries and hops, was selected as a representative isometric virus. Initial tests carried out in Alister's laboratory were spectacularly successful, so Tony and I embarked on a comprehensive evaluation of the method.

The technology was fairly straightforward. The problems we had to face were more conceptual in nature. Serology was regarded by many plant virologists as more of an art form than a scientific technique, interesting as an adjunct to infectivity tests and determining physicochemical properties but not really trusted as a mainstream diagnostic tool. To bring about a radical change in attitudes would require that this new procedure be universally applicable, reliable, and extremely robust. Our concern was to describe experimental conditions that would work with any virus, rather than optimizing conditions for one or two; precise instructions would be needed so that the likelihood of failure due to experimental or operator error would be minimized. We held workshops and compiled and distributed a step-by-step operating protocol that rapidly found its way into laboratories in many countries. We were fortunate not to be encumbered by patents claims and controversy, which affected the more commercialized, highly competitive world of medicine. In the event the advantages of ELISA over classical precipitin-based serology became so manifestly self-evident that its acceptance as a leading immunodiagnostic procedure was inevitable and led in 1981 to my sharing in the American Phytopathological Society's Lee M. Hutchins Award with colleagues from Israel and the US.[1]

Although many variants[2,3] of ELISA have been described and its use extended to other plant pathogens,[4,5] it is gratifying to realize that the procedure as described a decade and a half ago is still in use today by scientists and plant-health agencies throughout the world.

1. **Bar-Joseph M, Garnsey S M, Gonsalves D, Moscovitz M, Purcifull D E, Clark M F & Loebenstein G.** The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. *Phytopathology* 69:190-4, 1979. (Cited 45 times.)
2. **Clark M F, Lister R M & Bar-Joseph M.** ELISA techniques. (Weissbach A & Weissbach H, eds.) *Methods for plant molecular biology*. London: Academic Press, 1988. p. 507-30.
3. **Koenig R.** ELISA in the study of homologous and heterologous reactions of plant viruses. *J. Gen. Virol.* 40:309-18, 1978. (Cited 95 times.)
4. **Kishinevsky B & Gurfel D.** Evaluation of enzyme-linked immunosorbent assay (ELISA) for serological identification of different rhizobium strains. *J. Appl. Bacteriol.* 49:517-26, 1980. (Cited 15 times.)
5. **Clark M F, Morton A & Buss S L.** Preparation of mycoplasma immunogens from plants and a comparison of polyclonal and monoclonal antibodies made against primula yellows MLO-associated antigens. *Ann. Appl. Biol.* 114:111-24, 1989.

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