

The ELISA, Enzyme-Linked Immunosorbent Assay

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Featured Article: Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971;8:871–4.²

This paper was my first as a graduate student of Professor Peter Perlmann at Stockholm University. At the time, RIAs were in full bloom, but they were too sophisticated for many areas of research and diagnosis because they required expensive equipment and used antigens and antibodies labeled with radioactive isotopes with short half-lives. We wanted something simpler with the same sensitivity. Techniques for labeling antibodies with enzymes had been described for immunohistochemistry (1), and we thought they could be useful for serologic assays as well. First, we gave the prospective enzyme immunoassay a name: enzyme-linked immunosorbent assay, or ELISA (an important decision). Second, we chose to use an enzyme for which there was, at the time, a soluble and sensitive substrate-product system, namely alkaline phosphatase. Third, we used the procedure of a standard RIA, i.e., a competitive assay in which labeled antigen competed with antigen in the sample for binding to antibodies covalently coupled to cellulose particles. Separation of antibody-bound antigen from free antigen was done by repeated centrifugation and washing. We had the first ELISA calibration curve in early 1970. As usual, it took a while to find a journal willing to publish the result. We were continually asked why anyone would want to measure rabbit IgG! Of course, the ELISA was meant to be a proof-of-concept assay. Nevertheless, most rejections stated that the paper contained nothing new.

I think the very next developments were crucial in making the ELISA into what it is today. The first was to use the technique of coating plastic with antigens or antibodies to make a solid phase/immunosorbent (2). Washing of the solid phase then became extremely simple. It is amazing how predictably and reproducibly one can absorb a protein onto a polystyrene surface, and how relatively durable the absorption is. The next development was the introduction of microtiter plates

as the assay format (3). A standardized format allowed the subsequent development of all sorts of gadgets for the easy execution of assays in research and diagnostic laboratories, including multichannel pipettes, washing devices, and color and fluorescence plate readers. Then came monoclonal antibodies—a match for ELISA made in heaven, particularly for sandwich assays for measuring antigens (4). For many years, numerous companies have sold a wide variety of antibodies, enzyme-labeled reagents, coated 8-well strips, and kits to facilitate research in many areas.

For the rest of my research career, I used the ELISA in my own laboratory for all sorts of applications (5), but I was not involved in further assay development. It has been very rewarding to watch from the sidelines how the ELISA has been used in numerous important diagnostic tests and how it has created a multibillion-dollar industry. My favorite ELISAs are the quick and easy ones that cannot be done any other way. For example, the organization Direct Relief International has performed over 7 million tests for HIV in developing countries with an extremely simple kit (Abbott Determine), manufactured and donated by Abbott Laboratories. The main goal is to identify pregnant women infected with HIV to prevent mother-to-child transmission during delivery. Animal health is another area in which the ELISA has been and still is important. A leading diagnostic company in the area of animal diseases (IDEXX Laboratories), with billions of dollars in revenue, has over 80% of its business in the diagnosis of diseases in dogs and cats; a large portion of that is in simple and fast ELISAs.

After all this time, it is surprising that ELISA has not been replaced by something more modern, such as the PCR, chemiluminescence, or something else. The reason? Few assay systems are as simple as the ELISA and require so little in terms of automation and equipment. There is beauty in simplicity.

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