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Engvall E & Perlmann P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *J. Immunology* 109:129-35, 1972.
[Wenner-Gren Inst., Dept. Immunology, Univ. Stockholm, Sweden]

The paper describes a solid-phase immunoassay for quantitation of antibodies. The antigen is adsorbed to the assay tube and enzyme-labeled anti-immunoglobulin is used for detection of bound antibodies. [The *SCI*® indicates that this paper has been cited in over 1,545 publications.]

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In 1969 I entered a graduate program at the Department of Immunology of the University of Stockholm, Sweden. The project assigned to me by my supervisor, Peter Perlmann, was to develop a quantitative enzyme immunoassay. At that time, radioimmunoassays were in full bloom, but we believed that nonisotopic techniques of comparable sensitivity would potentially be of great utility. A technique for labeling antibodies with enzymes for the purpose of immunohistochemistry had been published.[1] Our contribution was to develop a technology for the use of enzyme-labeled antigens as well as antibodies in quantitative assays.

In early 1970 we had the first quantitative enzyme immunoassay working, and we had also decided on a name for it: ELISA (enzyme-linked immunosorbent assay). The first assays used cellulose as a particulate immunoadsorbent, which required repeated and boring centrifugations in all of the washing steps. We were, therefore, looking for ways to simplify the assay. After coming across the paper by Catt and Tregear,[2] we were quick to explore and optimize the capacity of proteins to stick to plastic and to use tubes coated with antibodies and antigens as immunoadsorbents for ELISA. This application resulted in a simple, robust, inexpensive, and almost foolproof test system,[3] which has become very popular. An exciting trip to East Africa immediately after my thesis defense was arranged by Alister Voller of the Zoological Society of London and proved that ELISA, because of its simplicity, could be used even under primitive conditions.

ELISA has been used in many different areas of research, development, and practice and has spurred a multimillion-dollar industry. A particularly gratifying application from my personal point of view was the ELISA for feline leukemia virus antigen.[4] As a breeder of Abyssinian cats in my spare time, this is one important ELISA that I and other cat breeders use routinely to monitor our breeding colonies.

After my degree, I went to work with Erkki Ruoslahti, first in Finland and then in California. In our research, ELISA was important in the discovery of the affinity of fibronectin for collagen.[5] This led to the simple procedure for purification of fibronectin by gelatin-Sepharose affinity chromatography and a boost for fibronectin and extracellular matrix research. The two-site, one-step assay using monoclonal antibodies[6] showed that the ELISA technology and monoclonal antibodies were well suited to each other.

The many and varied applications of ELISA have resulted in numerous citations. However, because of the many different types of applications and slight individual modifications in procedures, our paper has only received citations in a fraction of the thousands of papers published each year on ELISA.

On the other hand, I cannot complain about lack of attention. In 1976 Perlmann and I, together with van Weemen and Schuurs,[7] were awarded the prize in analytical biochemistry by the German Society for Clinical Chemistry, and in 1986 Perlmann and I received the Smith Kline Bio-Science Laboratories award from the Clinical Ligand Assay Society.

1. **Avrameas S.** Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* 6:43-52, 1969. (Cited 1,140 times.)
2. **Catt K & Tregear G W.** Solid-phase radioimmunoassay and antibody-coated tubes. *Science* 158:1570-2, 1967. (Cited 535 times.)
3. **Engvall E.** Enzyme immunoassay—ELISA and EMIT. *Meth. Enzymology* 70A:419-39, 1980. (Cited 320 times.)
4. **Lutz H, Pedersen N C, Harris C W, Higgins J & Theilen G H.** Detection of feline leukemia virus infection. *Feline Pract.* 10:13-23, 1980. (Cited 20 times.)
5. **Engvall E & Ruoslahti E.** Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. *Int. J. Cancer* 20:1-5, 1977. (Cited 1,105 times.)
6. **Uotila M, Ruoslahti E & Engvall E.** Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alphafetoprotein. *J. Immunol. Method.* 42:11-5, 1981. (Cited 65 times.)
7. **van Weemen B K & Schuurs A H W M.** Immunoassay using antigen-enzyme conjugates. *FEBS Lett.* 15:232-6, 1971. (Cited 320 times.)

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