

Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA)

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This brief note addresses the historical background of the invention of the enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA). These assays were developed independently and simultaneously by the research group of Peter Perlmann and Eva Engvall at Stockholm University in Sweden and by the research group of Anton Schuurs and Bauke van Weemen in The Netherlands. Today, fully automated instruments in medical laboratories around the world use the immunoassay principle with an enzyme as the reporter label for routine measurements of innumerable analytes in patient samples. The impact of EIA/ELISA is reflected in the overwhelmingly large number of times it has appeared as a keyword in the literature since the 1970s. Clinicians and their patients, medical laboratories, in vitro diagnostics manufacturers, and worldwide healthcare systems owe much to these four inventors.

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Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) have become household names for medical laboratories, manufacturers of in vitro diagnostic products, regulatory bodies, and external quality assessment and proficiency-testing organizations. This brief historical note spotlights the development of enzyme labels in immunoassay from the invention of this method in the 1960s through its development and early use during the 1970s and 1980s.

The first published EIA and ELISA systems differed in assay design, but both techniques are based on the principle of immunoassay with an enzyme rather than radioactivity as the reporter label. Two scientific research groups independently and simultaneously developed this idea and executed the necessary experiments to demonstrate its feasibility. The ELISA technique was conceptualized and developed by Peter Perlmann, principal inves-

tigator, and Eva Engvall at Stockholm University, Sweden, and the EIA technique by Anton Schuurs, principal investigator, and Bauke van Weemen at the Research Laboratories of NV Organon, Oss, The Netherlands.

RIA was first described in 1960 for measurement of endogenous plasma insulin by Solomon Berson and Rosalyn Yalow of the Veterans Administration Hospital in New York (1). Yalow would later be awarded the 1977 Nobel Prize for Medicine for “the development of the RIA for peptide hormones” (2), but because of his untimely death in 1972, Berson could not share the award. Also in 1960, Dr. Roger Ekins of Middlesex Hospital in London published his findings on “saturation analysis” used to estimate thyroxine in human plasma (3).

The immunoassay technique with a radioactive label immediately caught the imagination of many researchers and clinicians, and in the ensuing decade RIAs for new analytes were published at a rapid pace and variants of the method were rapidly developed. In 1968, Miles and Hales published their first results of an “immuno-radiometric” technique with radioactive labeled antibodies rather than labeled antigen for measuring insulin in human plasma (4).

In many laboratories around the world, special facilities were built in which investigators could safely work with the amounts of radioactivity required for the labeling of antigens or antibodies, but concern persisted with regard to the safety of laboratory personnel, the radioactive waste problem, the requirements of building special laboratory facilities, and the procurement of expensive counting equipment. It should be recalled that in the original studies (1, 3, 4) iodine-131 (β and γ radiation) was used for the labeling because no alternatives were available at that time. The potential health problems related to the use of radioactive materials were greatly diminished when manufacturers such as Amersham and NEN began marketing iodine-125 (weak γ radiation) preparations of sufficiently high specific activity and purity.

At meetings, such as the ERIAC (European RadioImmunoAssay Club) in Basel in the early 1970s, the idea of

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using enzyme labels was met with skepticism and incredulity. How could so bulky and large a molecule as an enzyme be attached to an antigen or antibody without sterically hindering the immunochemical reaction between antigen and antibody? This objection on principle was nullified by carefully planned and executed experiments to demonstrate the feasibility of enzyme assays. Initial results were encouraging, and later the resounding success of the enzyme-(linked) immunoassay technique proved all skeptics wrong.

How did Perlmann and Schuurs each invent a method that others found inconceivable? These two principal investigators, when personally contacted by this author, could not report an anecdote about a particular or spectacular moment of insight. Instead, the classic pattern of research was followed, building on results published by colleagues in other fields, notably Stratis Avrameas of Villejuif, France (5, 6), G.B. Pierce of Los Angeles, California (7), and L. Wide of Uppsala, Sweden (8).

Between 1966 and 1969, the group in Villejuif reported their successful results of coupling antigens or antibodies with enzymes such as alkaline phosphatase (EC 3.1.3.1), glucose oxidase (EC 1.1.3.4), and others (5, 6). Avrameas and colleagues (5, 6) described the optimal coupling of these molecules by means of glutaraldehyde. Their purpose was to use the enzyme-labeled antigens and antibodies to detect antibodies or antigens by immunofluorescence, and they applied their tools to histopathology. In Los Angeles, Pierce and colleagues (7) had successfully developed the same line of research, also for histochemical purposes. The Uppsala group had developed a so-called (radio)immunosorbent technique in which antibodies were insolubilized by coupling them to cellulose or Sephadex beads.

Engvall and Perlmann published their first paper on ELISA in 1971 (9) and demonstrated quantitative measurement of IgG in rabbit serum with alkaline phosphatase as the reporter label. In the same year, van Weemen and Schuurs (10) published their innovative work on EIA and reported that it was possible to quantify human chorionic gonadotropin concentrations in urine. They used the enzyme horseradish peroxidase (EC 1.11.17), coupled by means of glutaraldehyde, as the reporter label. The Schuurs group secured patents on their findings [US patent application 762120, filed September 24, 1968 (11); Dutch patent applications 7016396, filed November 10, 1970, and 7018838, filed December 28, 1970 (12)].

Perlmann's further research included cytotoxicity of human lymphocytes (13) and immunogen selection and epitope mapping for malaria vaccine development (14). Engvall's group applied the ELISA measurement tool to parasitology [e.g., malaria (15) and trichinosis (16)], microbiology (17), and oncology (18–20). Engvall then focused her scientific interests on the biochemistry of tissues, e.g., fibronectin, laminin, integrins, and muscular dystrophies. Engvall's laboratory is currently investigating the use of differentiation factors for muscle regenera-

tion and myogenic cells from nonmuscle tissues for muscle cell replacement (21).

During the late 1960s and early 1970s, many RIA test systems were essentially "home-brew" methods developed by individual researchers who could not keep pace (particularly financially) with the possibilities and facilities of commercial manufacturers such as Boehringer-Mannheim (Germany), Abbott (United States), and Organon Teknika (The Netherlands), to name only a few. Commercialization of EIA/ELISA test kits had started. Solid-phase techniques (8, 22) were used in the development of microtiter plates (96 wells) in which either an antigen or an antibody is noncovalently bound to a solid-phase support. Technical advances led to automated pipetting devices (Micromedics; Hamilton), multichannel pipettes (Lab Systems), and microtiter plate readers and washers (Fig. 1), and in the 1980s fully automated test instruments were manufactured by Boehringer-Mannheim and Abbott, among others. Such automated systems have come to stay in medical laboratories.

The spectacular invention EIA/ELISA generated a whole series of test formats, from the immunoenzymometric [already mentioned in Ref. (4)] to the many variants of "sandwich" test procedures. For a comprehensive review of the possibilities the reader is referred to Ref. (23). The Dutch group at Organon/Organon Teknika successfully developed EIA systems in the field of reproductive endocrinology, including assays for human chorionic gonadotropin (10, 24), total estrogens, and human placental lactogen (25) in plasma. However, the new tests did not become commercially successful until the late 1970s and early 1980s, when they matched the exquisite sensitivity of existing RIA systems for the same analytes.

In the early 1970s, blood-bank screening for virologic diseases such as hepatitis B antigen was done either by (semi)automated RIA or nonradioactive but rather cumbersome hemagglutination tests. In 1976, Organon Teknika developed and marketed a highly successful EIA system for the hepatitis B surface antigen (HbsAg) (26), featuring a 96-well microtiter plate format. This test became the first commercially available EIA (Fig. 2).

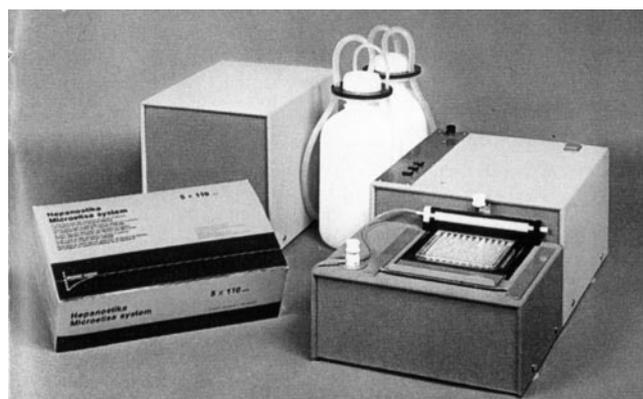


Fig. 1. Washer for the HEPANOSTIKA from a manufacturer's brochure, "5 Years of Organon Teknika", published in 1977.



Fig. 2. Promotional photo of the first EIA, the HEPANOSTIKA test kit by Organon Teknika (The Netherlands).

Other microbiological and virologic diagnostic tests soon followed, e.g., for hepatitis B “e” (HBe) antigens (27), rubella antibodies, toxoplasma antibodies, and in the 1980s, an EIA system for detection of human immunodeficiency virus antibodies.

The impact of diagnostic immunoassays, be they RIA, EIA, or ELISA, on patients, clinicians, and the healthcare system in general is virtually unsurpassed. To substantiate this subjective statement, this author searched PubMed with the search terms “enzyme-immunoassay”, “enzyme-linked immunoassay”, and “RIA”, in clusters of 5 years from 1960 to 2005. The estimates of the number of articles quoting these keywords are given in Fig. 3. The sheer numbers are astounding! The peak of RIA quotations seems to have occurred between 1980 and 1990. The number of citations decreased from 1990 to 2000, but is still quite substantial. The number of articles with EIA or ELISA as a keyword increased rapidly in the 1980s and

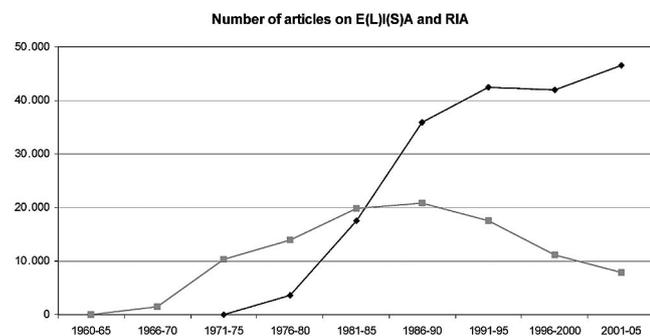


Fig. 3. Estimates of the number of articles published per 5-year period from 1960 to 2005.

The search was done in February 2005 in PubMed/National Library of Medicine, NIH, with the search terms: enzyme-immunoassay, enzyme-linked immunosorbent assay (EIA/ELISA combined), and RIA. (*Ordinate*), number of articles in which the keywords are quoted. (*Abscissa*), 5-year periods from 1960 to 2005. ◆, combined EIA/ELISA; ■, RIA. [Note: I do not pretend that the numbers in this figure are precise; the trends, however, are evident.]



Fig. 4. Preis Biochemische Analytik, Munich, April 1976.

From left to right, Dr. Eva Engvall (Sweden), Dr. Anton Schuurs (The Netherlands), Dr. Peter Perlmann (Sweden), Dr. Bauke van Weemen (The Netherlands), and Prof. Johannes Büttner (Germany), President of the German Society of Clinical Chemistry (Deutsche Gesellschaft für Klinische Chemie; DGKC).

plateaued at an amazing ~40 000 quotations per 5 years in the 1990s. A decrease in this trend is not yet in sight.

In conclusion, the number of analytical and clinical investigations relying on these measurement procedures worldwide is exceedingly large. Thus, one can imagine that the numbers of measurements and determinations using immunoassay for routine patient care are astronomical. The clinical impact of EIA/ELISA as nonradioactive variants of immunoassays is indeed overwhelming. Perlmann, Schuurs, Engvall, and van Weemen were honored for their inventions when they received the German scientific award of the “Biochemische Analytik” in 1976 (Fig. 4), 5 years after they had published their first papers. Given the impact that their inventions have had on clinical diagnosis and healthcare in general, as well as on the development of a well-established *in vitro* diagnostic industry, these inventors deserve to be honored again.

During submission of this historical note for manuscript review, the sad news arrived that Dr. Perlmann had died on April 19, 2005, in Stockholm. He had received the submitted draft of this paper, however, in March 2005.

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