OpenAccessPub

Journal of Proteomics and Genomics Research

ISSN: 2326-0793

DOI: 10.14302/issn.2326-0793.jpgr-21-3917

Mini Review

Freely Available Online

The Evolution of the Enzyme Immunoassay/Enzyme-Linked Immunosorbent Assay

Leonid Tarassishin[1,*]

[1]Department of Pathology, Albert Einstein College of Medicine, United States.

Corresponding author:

Leonid Tarassishin, Department of Pathology, Albert Einstein College of Medicine, United States.

Keywords:

Enzyme immunoassay, ELISA, evolution, specificity

Received: Jul 30, 2021

Accepted: Aug 11, 2021

Published: Aug 16, 2021

Editor:

Palaniappan Ramasamy, Director-Research, Sree Balaji Medical College and Hospital, Chennai, BIHER, Tamilnadu, India.

Abstract

50 years ago the Enzyme Immunoassay Enzyme-Linked Immunosorbent Assay, mostly known as ELISA was developed. This is a powerful but simple method that is very widely used in the diagnostic practice, as well as in biomedical research. During this time a number of ELISA modification were developed that significantly increased its properties, especially the senstivity, such as avidin-biotin assay, immuno-PCR, nano-ELISA and finally, the digital ELISA. This short review describes the principles of ELISA and the evolution from a conventional assay to the modern ultra-sensitive method.

Most of the immunological methods have two components: antigen and antibody. The high specificity of their interaction gives a possibility to detect one of them if other one is included in the reaction as a specific partner. The simplest method for antigen detection in the presence of the antibody is immune diffusion (radial immune diffusion in that case), which practically the formation of precipitate of the “antigen-antibody” complex, when the target antigen diffuses from well into agarose containing the specific antibody. Unfortunately, this assay, as well as other traditional methods, like hemagglutination or complement fixation, have a low sensitivity and are unwieldy.

A significant next step was created to overcome this problem. It came from an idea to use the labeled antigens, or antibodies. In 1960 Rosalyn Yalow and Solomon Berson described the new method for detection of the endogenous plasma insulin with a radiolabeled antigen [1]. This method was called RadioImmunoAssay (RIA). Then in 1971 two papers from two independently working research groups of Peter Perlman and Eva Engvall in Sweden and Anton

Schuurs and Bauke Van Weemen in the Netherlands appeared simultaneously. Using the principles of RIA they developed a new method by conjugating the target antigen (or antibody) with enzyme instead the radioactive iodine 125 [2, 3, 4]. It became the ELISA, Enzyme-Linked Immunosorbent Assay.

Shortly after development ELISA became widely accepted for the antigens and antibodies detection and quantification in human and veterinary medicine, agriculture, environment monitoring, and biomedical research. There are significant applications ELISA for diseases diagnosis and control of treatment [6]. The most well-known examples are diagnosis of AIDS through the detection of the antibody for HIV and the detection SARS-CoV-2 antibody as response to the Covid-19 infection or to a specific vaccine.

Besides the enzyme-labeled antigen or antibody this method requires a solid phase, where a 96-well microtiter plate made of rigid polystyrene, polyvinyl or polypropylene can be used. The enzymes include: beta-galactosidase, glucose oxidase, and, more frequently used, alkaline phosphatase or horseradish peroxidase. Depending on the substrate, the final product can be registered visually (change the color of solution) or with a spectrophotometer/microplate reader/, or through either chemiluminescence (using luminometer) or fluorescence (using fluorometer) [5]. There are 4 major types of ELISA: direct, indirect, sandwich, and competitive [6, 7]. In the **direct ELISA** the solid phase (microtiter plate) is coated with an antigen or antibody, and then the enzyme-conjugated antibody or antigen, accordingly, is incubated to form the "antigen-antibody" complex. A disadvantage of this assay is that it is necessary to label the antigen or antibody every time. This problem was overcome by the **indirect ELISA** by including a secondary enzyme-conjugated antibody. In the **sandwich ELISA** the same specific antibody is used for coating the microplate wells (capture antibody) and for the detection of the antigen (detection antibody), but last one is in the enzyme-labeled form. Thus, antigen will be trapped between two specific antibodies (Fig. 1A). Another version of this method is the **indirect sandwich ELISA.** In this case specific antibodies from different species such as mouse or rabbit are used. Then, at the next step the secondary antibody (anti-rabbit, particularly), which conjugated with the enzyme is added and the reaction continues by incubation, washing to remove the unbound materials, adding the substrate for the enzyme (for example, the p-nitro-phenyl phosphate for alkaline phosphatase or the

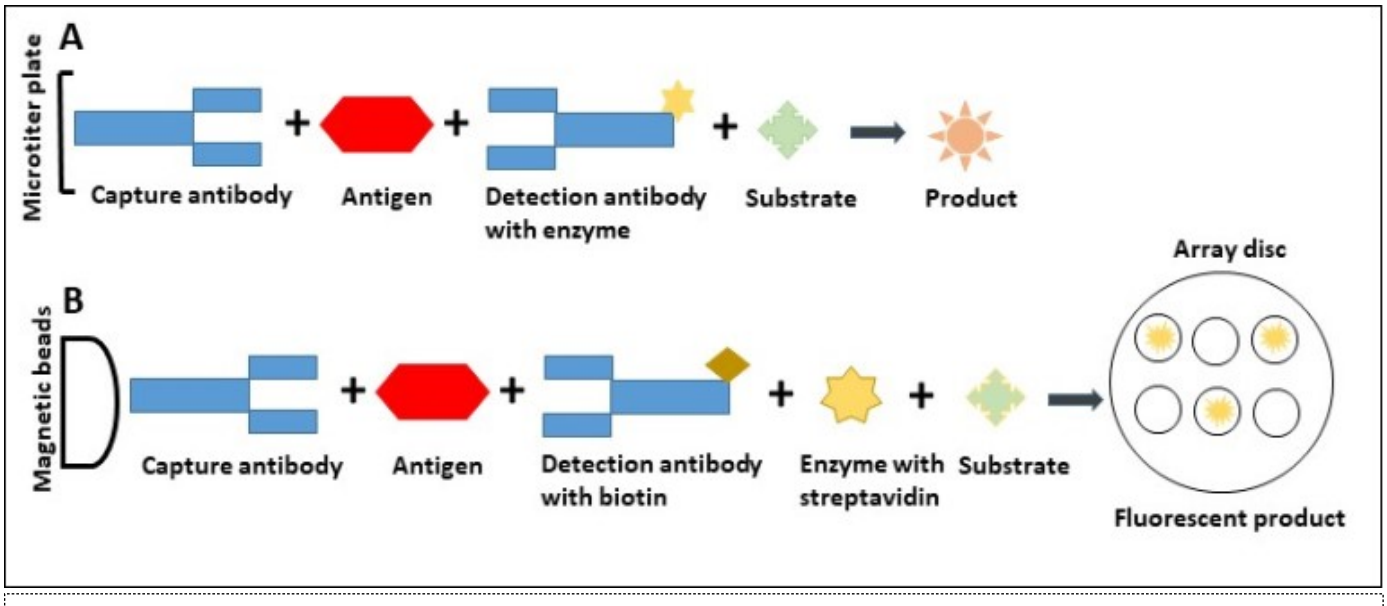

Figure 1. Scheme of conventional sandwich ELISA (A) and digital ELISA (B)

tetramethylbenzidine with hydrogen peroxidase for horseradish peroxidase), and then stopping the reaction. The result is estimated (and quantified) by a microplate reader. In the **competitive ELISA** the microtiter plate wells are coated with a specific antibody or a specific antigen. Then, the enzyme-conjugated antigen or antibody is simultaneously tested and placed into wells to compete with each other for binding to the antibody or antigen, accordingly.

At present, ready-to-use ELISA kits are commercially available but it still requires optimization, which includes the preparation of samples (dilution in the appropriate buffer to the suitable concentration) and the determination of the optimal time for the enzyme-substrate reaction (it should be stopped at the maximum level of product). One of the ELISA disadvantage is the false positive/negative reactions. In some cases a false positive/negative can be avoided by using other (specific) antibodies, but in other cases it appears randomly and cannot be properly explained. Usually it requires a repeat analysis or validation with other methods. Home-made ELISA needs much more work. One should pay attention to the quality of assay plate, the blocking buffer (high quality bovine serum albumin), the capture and the detection antibodies, the target antigen (avoiding cross-reaction with similar proteins in the specimens), the enzyme conjugate, the substrate, the washes (freshly prepared buffer without any contamination) as well as the control of temperature (and time of incubation) and signal detection [8].

The specificity of the "Antigen-Antibody" interaction depends on the quality of the antigens and antibodies, but sensitivity requires different approaches.

Recently the avidin-biotin system has become very popular. It is based on the high affinity of avidin and biotin: dissociation constant (K_d) on the order of 10^{-14} mol/L. As result, the sensitivity is sufficiently increased (9). This system requires the conjugation biotin with a secondary antibody followed by an interaction with avidin (or streptavidin-avidin from bacteria *Streptomyces avidinii*), conjugated to the enzyme (or fluorochrome).

The highly sensitive method based on nanoparticles was proposed recently and called nano-ELISA. For example, this method was able to detect 5.7 pg/ml cancer biomarker p53 after 2 hours assay in compare with 0.125 ng/ml for conventional ELISA [10].

A new direction, which significantly increases sensitivity, became a combination of two methods, particularly ELISA and polymerase chain reaction (PCR) called immuno-PCR [11]. In this technique the antibody is labeled with a DNA fragment either directly or through a streptavidin-biotin conjugation followed an amplification by PCR. The quantitative immuno-PCR employs real-time PCR, which results in increased sensitivity (at least for 10 times), decreased reaction volume (as little as 10 microliters per sample) with high reproducibility and robustness [12]

A new era for ELISA came with the development of the digital ELISA (dELISA) [13, 14, 15]. dELISA is based on a Single Molecular Array (SiMoA) platform (Fig. 1B). The initial steps are similar to ones in the conventional indirect sandwich ELISA. Thus, the test antigen is incubated with paramagnetic beads, which are loaded with a captured antibody and a detection biotinylated antibody. Then the streptavidin-conjugated enzyme (streptavidin-β-galactosidase) is added. After that the fluorescent substrate is added and the beads are loaded into an array disc, which contains more than 200,000 microwells – each well can hold only one bead. The wells are sealed to restrict the fluorescent product of the enzyme-substrate reaction to a 50-fL volume. This produces a high local concentration of the fluorescent product. Then fluorescent images of the array are received to observe the fluorescent signal increase, which confirms the presence of the immunocomplex.

dELISA (SiMoA) is a super powerful new technique with the sensitivity that is approximately 1000 times higher than the conventional ELISA. It can detect

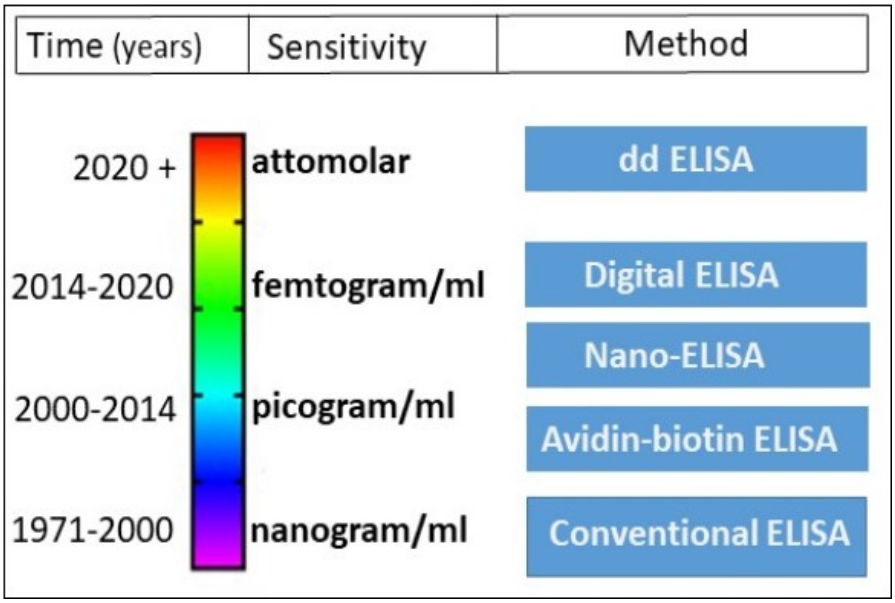

Figure 2. Evolution of ELISA

the proteins at an ultra-low (femtogram) level in a wide variety of biological samples. SiMoA has a multiplex capability and is fully automated by using Simoa HD-X Analyzed (Quanterix, MA, USA) [16].

A new recent modifications of dELISA (SiMoA) are the droplet digital enzyme-linked immunosorbent assay (dELISA) and the dropcast single molecule assay (dSimoa) [17, 18], which allow to increase the sensitivity of this method up to 25-fold and detect the attomolar protein concentration.

dELISA and its modifications allow to detect proteins that are present in biological specimens at very low level (single molecule), such as cancer or neurological biomarkers [13, 19, 20].

This year we celebrate 50 years of ELISA development. During this time we observed how quickly this method has become widely used in clinical practice and in the biomedical research. Eva Engvall recently wrote, "There seems to be no limit to the uses to which ELISA can be put!" [21]. At the same time we saw the various modifications of ELISA in terms of sensitivity (Fig. 2), flexibility, miniaturization, and automation. There is still room for improvement – just need new ideas and cooperative efforts.

References

1. Yalow R.S., Berson S.A. (1960) Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39 (7), 1157-1175.
2. Engvall E., Perlmann P. (1971) Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry. 8 (9), 871-874.
3. Van Weemen B.K., Schuurs A.H. (1971) Immunoassay using antigen-enzyme conjugates. FEBS Lett. 15 (3), 232-236.
4. Lequin R.M. (2005) Enzyme Immunoassay (EIA)/ Enzyme-Linked Immunosorbent Assay (ELISA). Clin. Chem. 51 (12), 2415-2418.
5. Hornbeck P. (1991) Enzyme-linked immunosorbent assay. Curr. Protoc. Immunol. By John Willey and Sons, Inc. Chapter 2, 2.1.1-2.1.22.
6. Tarasishin L. (1986) Immunoenzyme analysis and its modifications in the study of viral antigens. Mikrobiol. Zh. 48 (4), 99-104.
7. Aydin S. (2015) A short history, principles, and types of ELISA, and our laboratory experience with

peptide/protein analyses using ELISA. Peptides. 72, 4 -15.

8. Hosseini S., Varquez-Villeges P., Rito-Palomares M., Martins-Chopa S.O. (2017) Advantages, disadvantages and modifications of conventional ELISA. In: Enzyme-linked immunosorbent assay (ELISA); Ed: Kumar A., Rao A.A. Springer: Singapore; p. 67-115.
9. Kendall C., Ionescu-Matiu I., Dreesman G.R. (1983) Utilization of the biotin/avidin system to amplify the sensitivity of the enzyme-linked immunosorbent assay (ELISA). J. Immunol. Meth. 56 (3), 329-339.
10. Jia C-P., Zhong X-Q, Hua B. et al. (2009) Nano-ELISA for highly sensitive protein detection. Biosens. Bioelectron, 24 (9), 2836-2841.
11. Sano T., Smith C.L., Cantor C.R. (1992) Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates. Science. 258 (5079), 120-122.
12. Niemeyer C.M., Adler M., Wacker R. (2007) Detecting antigens by quantitative immune-PCR. Nat. Protoc. 2 (8), 1918-1930.
13. Rissin D.M., Kan C.W., Campbell T.G. et al. (2010) Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat. Biotechnol. 28 (6), 595-599.
14. Kan C.W., Rivnak A.J., Campbell T.G. et al. (2012) Isolation and detection of single molecules on paramagnetic beads using sequential fluid flows in microfabricated polymer array assemblies. Lab Chip. 15 (5), 977-985.
15. Cohen L., Walt D.R. (2017) Single-molecule arrays for protein and nucleic acid analysis. Ann. Rev. Anal. Chem. 10 (1), 345-363.
16. Wilson D.H., Rissin D.M., Kan C.W. et al. (2016) The Simoa HD-1 analyzer: A novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing. J. Lab. Autom. 21 (4), 533-547.
17. Cohen L., Cui N., Cai Y. et al. (2020) Single molecule protein detection with attomolar sensitivity using droplet digital enzyme-linked immunosorbent assay. ACS Nano. 14 (8), 9491-9501.
18. Wu C., Garden P.M., Walt D.R. (2020) Ultrasensitive detection of attomolar protein concentrations by dropcast single molecule assays. J. Am. Chem. Soc. 142 (28), 12314-12323.
19. Thangavelu B., Boutte A.M. (2021) Single molecule assay for ultrasensitive detection of Cathepsin B in human blood. ACS Omega. 6 (14), 9609-9616.
20. Li D., Mielke M.M. (2019) An update on blood-based markers of Alzheimer's disease using the SiMoA platform. Neurol. Ther. 8 (Suppl. 2), S73-S82.
21. Engvall E. (2005) Perspective on the historical note on EIA/ELISA by Dr. R.M. Lequin. Clin. Chem. 51 (12), 2225.