

Paper based Vs conventional enzyme linked immunosorbent assay: a review of literature

Abstract

This brief note addresses the comparison between conventional ELISA and a slightly newer, equally sensitive, specific, less time consuming and cost effective version of its "Paper based ELISA," which have a few major benefits on the conventional ELISA.

Keywords: enzyme-linked immunosorbent assays, radioimmunoassay, enzyme immunoassay, paper microzone plates

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Abbreviations: DIA, dot-immunobinding assays; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; RIA, radio immuno assay

Introduction of ELISA

Enzyme-linked immunosorbent assays (ELISA) are widely used as diagnostic tools in medicine as well as quality control measures in various industries. They are also used as analytical tools in biomedical research for the detection and quantification of specific antigens or antibodies in a given sample. ELISA is actually derived from a previously known technique called radio Immuno Assay (RIA). Radioimmunoassay (RIA) was first described by Yalow and Berson in 1959, a great discovery for which they won a Nobel Prize in Medicine in 1977.¹ In search for replacing radioactive isotopes, ELISA was introduced in the 1970s.² Currently, many ELISA techniques are in use in various fields of science and technology. In the typical double antibody sandwich ELISA, antibody attached to the bottom of a well provides both antigen capture and immune specificity, while the second antibody linked to an enzyme provides detection and an amplification factor. Additional advantages of ELISA include the fact that results are highly quantitative and generally reproducible.³ At the same time, several weaknesses have been recognized for this method because ELISA performance is largely dependent on antibody quality, kit manufacturer's error, as well as operator skills and experience dealing are of keen importance.⁴ Enzyme-linked immunosorbent assay (ELISA) is routinely performed on 96-well plates to allow samples to be tested singly or in duplicate. This assay is fairly simple, requiring only a small amount of serum and is completed within a day, allowing the analysis of a large number of samples in a relatively short time.⁵

Principal of conventional ELISA

Enzyme Immunoassay (EIA) and ELISA uses the basic immunology concept of an antigen binding to its specific antibody,

which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample. EIA and ELISA utilize enzyme-labeled antigens and antibodies to detect the biological molecules. The most commonly used enzymes are alkaline phosphatase and glucose oxidase. The antigen in fluid phase is immobilized, usually in 96-well microtiter plates allowing the antigen to bind to its specific antibody, which is subsequently detected by a secondary, enzyme-coupled antibody. A chromogenic substrate for the enzyme yields a visible color change or fluorescence, indicating the presence of antigen. Quantitative or qualitative measures can be assessed based on such colorimetric reading. Fluorogenic substrates have higher sensitivity and can accurately measure levels of antigen concentrations in the sample.⁶

ELISA types and their sensitivity and specificity

The comparison between different types is given below in the Table 1.

Paper based ELISA

Porous membranes, including nitrocellulose and filter paper, have been used for decades in dot-immuno binding assays (DIA).¹²⁻¹⁴ Though DIAs are the simplest form of immunoassays on paper, they typically require one piece of nitrocellulose strip for each assay; the pieces of nitrocellulose have to be processed individually in Petri dishes, and the assays take several hours to complete.¹³ DIAs is typically qualitative, and provides only "yes/no" results¹⁵ but quantitative DIAs has also been reported.¹⁶ Paper based ELISA was first introduced by White strides Research Group from Harvard who found the filter paper can be used for immunoassay for recognition of antigen and antibody.¹⁷

Requirements for conventional and paper based ELISA

Conventional ELISA is usually performed in 96-well plates (fabricated by injection molding in plastic) which are quantitative in nature and well-suited for high-throughput assays. But the drawback includes requiring large volumes (20-200 μ L) of analytes and reagents for each assay, the time required for incubation and blocking (\geq 1 hour per step, because the reagents must diffuse to the surface of the wells), and the results are usually quantified using a plate reader instrument which typically costs over a \$20 000.¹³

Paper microzone plates for ELISA is almost same as conventional ELISA except it uses paper based 96-well plates prepared via wax printing method. In this microzone paper based wells only about 3 μ L of sample is required, and the results can be measured using a desktop scanner, costing a \$100 instrument only. In addition, the time required to complete an entire P-ELISA is less than one hour. This ease of fabrication of paper microzone plates also opens opportunities for a wide range of non-standard formats, and customized connections to carry reagents between zones¹⁸ (Table 2).

Table 1 Comparison among different conventional ELISA techniques on basis of screening agent, sensitivity and specificity

Types of ELISA	Screening	Sensitivity	Specificity
Direct ^{7,8}	Antibody	Low	Very High
Indirect ⁹	Antigen/antibody	High	Low
Sandwich ¹⁰	Antigen	Very high	Low/high
Competitive ¹¹	Antibody	High	High

Table 2 Steps for conventional and paper based ELISAs

Steps involved	Conventional	Paper based
Plate type used	96 wells polystyrene plate	96-microzone paper plate
Antigen immobilization	Pre immobilized	Self-immobilization
Test Sample	Mostly serum	Serum/ lesional fluid
Secondary antibody	May or may not be used	2 ^o Ab conjugated HRP
Washing fluid	PBST	PBST
Substrate	TMB+H ₂ O ₂	TMB+H ₂ O ₂
Color development & scanning for results	Special optical ELISA reader	Commercial desktop scanner

Advantages of P-ELISA

We believe that the combination of ELISA and patterned paper will provide a useful new protocol for performing immunoassays. P-ELISA offers three principal advantages over conventional ELISA:

- i. It is more rapid.
- ii. It requires only small volumes (1-10 μ L) of samples and reagents.
- iii. It utilizes simple equipment: a pipette, a refrigerator for storing the reagents, and a scanner.

P-ELISA thus extends the range of application of ELISA, especially to small laboratories, and to developing countries. The most understandable current disadvantage of P-ELISA is that it is less sensitive than conventional ELISA. It may be possible to decrease the limit of detection for the P-ELISA by optimizing the assay to suppress the background signal.

Conclusion

In conclusion, P-ELISA combines the sensitivity and specificity of the ELISA with convenient, low cost and ease-of-use of paper-based platforms; P-ELISA (at its current state of development) is faster and less expensive than the conventional ELISA, but somewhat less sensitive.

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Conflict of interest

The author declares no conflict of interest.

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