ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, 5 SECTIO D 2002

Department of Clinical Immunology, University School of Medicine, Lublin
Zakład Immunologii Klinicznej Akademii Medycznej w Lublinie

PIOTR POŻAROWSKI

The principles of ELISA usage

Podstawy zastosowania testów ELISA

One of the most often used assays in modern immunological laboratory is Enzyme Linked ImmunoSorbent Assay (ELISA). It is characterised by high sensitivity, repeatability and specificity. These tests are easy and quick (usually 4-6 hours) to perform. Moreover, there is no need to have sophisticated equipment or labs with special conditions (e.g. prepared for radioisotopes). It is also very important that the cost of the test is reasonable [1]. The commercially available 96-well kits cost approximately 25,000 PLN. This means that the cost of a single measurement starts from about 25 PLN. Much more cost-efficient are complete sets of reagents so that one can perform all the tests by oneself (including monoclonal antibodies coating). The cost of such a set, suitable for e.g. 20 plates, is usually about 7,000 PLN. The most inexpensive, but very difficult to perform properly, are self-made ELISA.

ELISA is usually used to measure antigens (including not only cytokines, hormones or chemokines, but also such ones as drugs, chemicals, toxins or others) and antibodies (specific or not — e.g. titre of IgG_1) in suspensions. All kinds of suspensions are suitable (e.g. serum, culture supernatants, cerebral or peritoneal fluid) [2].

Proteins are the most often measured antigen using ELISA. Their DNA is transcripted to RNA and then RNA is translated to polypeptides. After intracellular modification the proteins are stored in cells and sometimes secreted outside them. During all these steps of protein synthesis the precise measurements of them could be done. Of course different methods depending on the step must be chosen. PCR and RT-PCR measurements must be performed to detect DNA or RNA. Western Blotting or flow cytometry assays should be applied for intracellular detection. Flow cytometry is also a suitable tool for measuring secretion of a single cell. The most often used test to measure the final products of the protein synthesis, i.e. proteins, in organ fluids, is ELISA. It is worth emphasising that for a good understanding of this synthesis process one needs to carry out several different tests. For example, detection of proteins inside cells does not mean that they secret them. Moreover, detection of the same proteins in blood does not mean that they act (e.g. they can be inactive) or that they are produced by the cells we expected. On the other hand, when the proteins are undetectable it does not mean that they are not produced. The test can be, for example, not sensitive enough or they are consumed by other cells in tissues.

The principle of ELISA is based on coloured immunoenzymatic reaction (enzyme immunoassay — EIA) introduced into laboratory practise in 1966. A few years later the principles of ELISA were described [3]. After so many years the principles of ELISA have not changed, but its range of usefulness, sensitivity and flexibility increased a lot. Now, ELISA can be divided in two basic groups: competition assays (i.e. antigen capture, antibody capture) and non-competition assays (i.e. antibody capture, sandwich assay) (Fig. 1). Nowadays, ELISA can be used not only to detect (qualitative tests) or measure (quantitative tests) the titre of e.g. antigen, like in the past, but thanks to the fact that new ELISA clones have been developed, new fields of

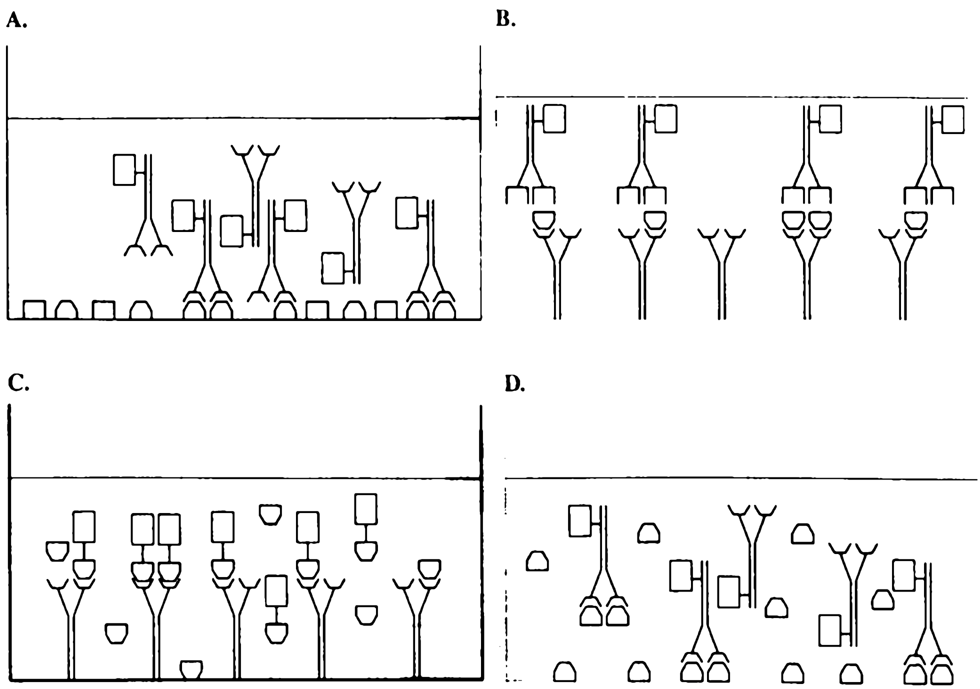

Fig. 1.
A. Non-competition antibody capture assay — samples (e.g. plasma or lysed bacteria) are used to coat the wells. The enzyme-linked specific antibodies are used to detect measured antigen. **B.** Non-competition sandwich assay — for details see Fig. 2. **C.** Competitive antigen capture assay — mixture of sample (measured antigen) and the same type antigen linked with an enzyme is added into antibody coated well. The coloured reaction depends on the ratio of measured/enzyme-linked antigen. **D.** Competitive antibody capture assay — the mixture of measured antigen and specific monoclonal antibodies linked with an enzyme is added into antigen coated well. The coloured reaction depends on the ratio of non-bound/bound antigen.

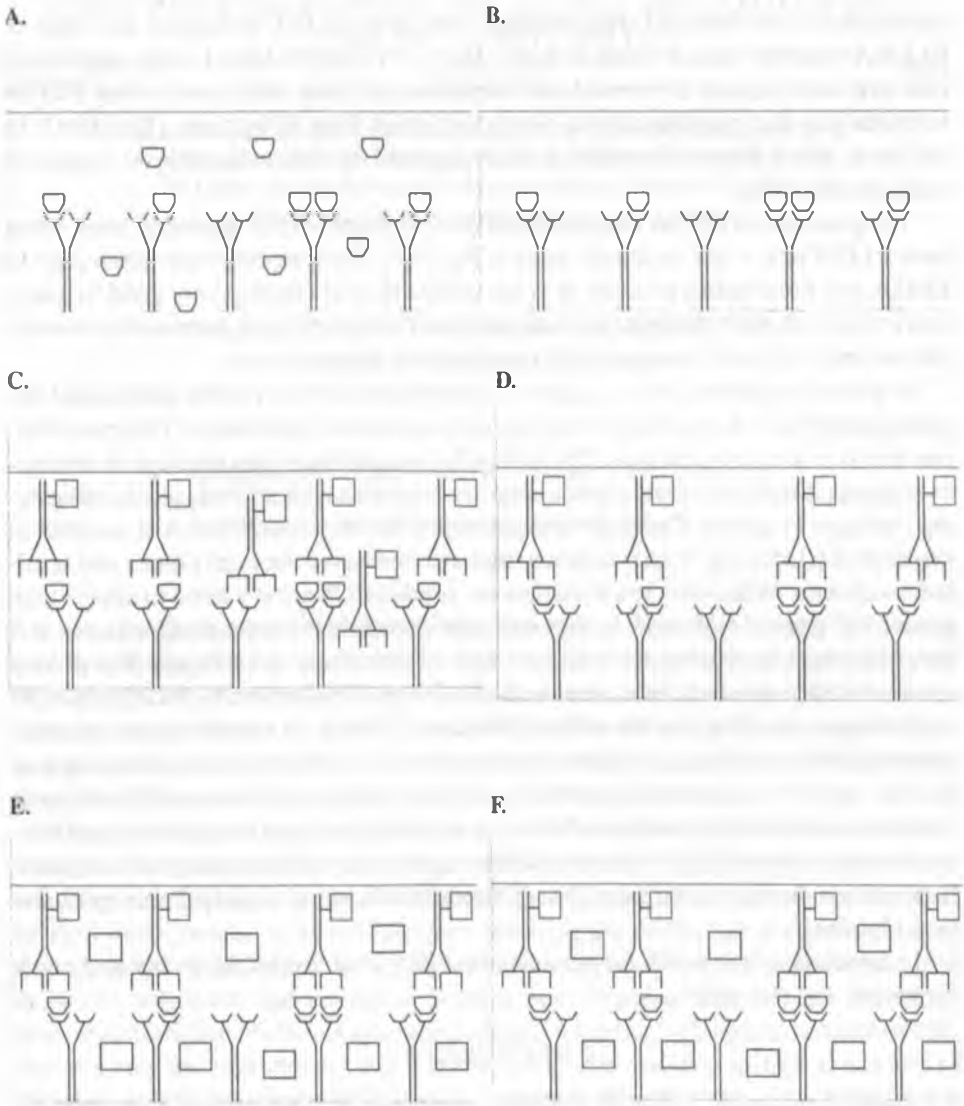

Fig. 2.

A. The solution of measured antigen (e.g. in serum sample) is added into the specific antibody coated well. **B.** After a different time of incubation the non-bound antigen is washed out. **C.** Antigen specific monoclonal antibodies linked to an enzyme are added (they must recognise "naked" epitops, different from the ones recognised by the antibodies adsorbed on the well). There is also the possibility of a two-step reaction using non-enzyme linked antibodies and then antibodies specific enzyme linked-antibodies. This type of reaction is more complicated, but more sensitive. **D.** Redundant antibodies are washed out. **E.** The enzyme substrate is added. **F.** The enzymes induce the concentration dependent coloured reaction which is measured in ELISA reader. In quantitative tests the antigen concentration is determined from standard curve. The establishment of it is made on standards measurements — measurements of wells with known concentration of antigen.

application have emerged. For example, the same ELISA principles are used in ELLISA (enzyme linked lectin sorbent assay) or in cell-ELISA. Lectins and whole cells are used instead of monoclonal antibodies in them. Moreover, using ELISA methods it is also possible now to detect the active form of cytokine (BioLISA). In this case, active forms of cytokines are recognised by their recombinant receptors bound to the wells.

The principle of ELISA was presented on an example of the, probably, most often used ELISA test — the sandwich assay in Fig. 2 [4]. The enzymes most often used in ELISA are: horseradish peroxidase (high sensitivity and rapidity, very good in qualitative tests), alkaline phosphatase (less sensitive then peroxidase, but results are more precise and repeated), biotyn-avidin (streptavidin, very sensitive).

It is very important for the patients if general practitioners really understand the principles of ELISA, especially its limitations and sources of mistakes. The most obvious mistakes are technical ones. They range from pipetting errors to usage of improper reagents. Most frequently experienced are problems with anticoagulants influencing coloured reaction. The most recommended one is heparin which is suitable in majority of ELISA kits. Other obvious rules are to observe the expiry dates and sensitivities of tests. When very low titrations are predicted, there is a need to apply "high sensitivity" tests. Unfortunately, they cost approximately twice the standard ones. It is very important to observe the standard time of defrosting the samples. The golden standard is that the sum of the time needed to freeze the sample and the time from its defrosting to pipetting into the wells is 30 minutes. It helps to avoid the problems with short half-life time of e.g. cytokines or chemokines. It is also worth emphasising that ELISA should be performed at least in doublets and approximately 20% of wells should be reserved for healthy reference group samples. And finally, there are mistakes in analysing the ELISA results. For example, detection of elevated titre of proinflammatory cytokines could be a reason of different from estimated inflammation site (e.g. sinusitis).

In conclusion it is worth emphasising that ELISA is a reliable, cheap and quick diagnostic and research tool.

REFERENCES

1. *Davies C.*: Concepts. In The Immunoassay Handbook. D. Wild, ed. New York, 1994, 83-115.
2. *Crowther J.R.*: ELISA. Theory and practice. Methods Mol. Biol., 1995; 42: 1-223.
3. *Engvall, E., Perlmann P.*: Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochem., 1971; 8: 871-874.
4. *Pathak S.S., van Oudenaren A., Savelkoul H.F.J.*: Quantification of immunoglobulin concentration by ELISA. In Immunology Method Manual, vol.2 I. Lefkovitz, ed. Academic Press, Inc., San Diego, 1997, 1056-1075.

STRESZCZENIE

Testy ELISA należą do najczęściej używanych metod diagnostyczno-badawczych. Charakteryzują się relatywnie niskimi kosztami, dużą powtarzalnością i dokładnością otrzymywanych wyników oraz krótkim czasem wykonania. W niniejszym artykule przedstawiono pokrótce zasadę działania, zalety oraz wady ELISA, jak również przydatne uwagi praktyczne.