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The Clinical Application of ELISA in Human Diseases

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Abstract. Enzyme-Linked Immunosorbent Assay (ELISA) has become one of the most widely used immunological detection technologies and plays an important role in the detection of human diseases. This paper reviews the research background, core position, types, basic principle, producer and the progress of the ELISA, and based on the principle and the procedure to research the clinical application of the ELISA in human diseases focusing on the tumor screening and detection, infectious diseases and allergy. Also analyse the strengths, weaknesses and the future prospect of the ELISA. The result of the research demonstrate that although there some limitation with sometimes have the rate of missed diagnosis and false diagnosis and some cannot be done simultaneously, it is a better way to develop some technology like the high-throughput ELISA technology, and we can also make the combination with ELISA and nucleic acid testing method. With high sensitivity, strong specificity, low cost and easy standardization, ELISA can serve as a powerful technical support for clinical detection and diagnosis of human diseases.

Keywords: ELISA, clinical diagnosis, cancer, infectious diseases, allergy

1. Introduction

Before the development of the Enzyme-Linked Immunosorbent Assay (ELISA) in 1971 [1], most immune assay used Radioimmunoassay (RIA) foe detection. However, there are some limitations of the RIA, which requires expensive and sophisticated equipment and in many small laboratories, RIA is rarely used because the rules for regulating radioactive isotopes are just too strict. So people have to search for another technology which enables sensitive and effective detection-ELISA [2]. In the past fifty years, ELISA has evolved from a research tool to a cornerstone of routine clinical diagnosis. ELISA is widely applied in clinical testing and laboratories, enabling accurate, reliable, convenient and sensitive quantification of various biomolecules. It is not only streamlining clinical workflows but also critical for facilitating timely patient recovery [3]. However, in the detection range, the traditional ELISA are being confined to the pico-to nanomolar, this limitation renders them significantly ineffective for the detection of rare or low -abundance biomarkers [4]. This technical limitation particularly significant in the early diagnosis of human diseases. For instance, certain cancer and low-grade viral infections and the concentrations of the relevant biomarker are often blow the detection threshold. The paper will analyse the basic principle and talk about the application of different types and procedure of ELISA in the current medical field. And based on

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this, analyze its advantages and disadvantages in application in human diseases. These analyses contribute to better prevention and clinical treatment of human diseases, and help improve public health levels.

2. The principles, types, and the procedure of the ELISA

2.1. The principles of ELISA

There are two basic principal of ELISA. The first one is the antigens and antibodies highly specific binding [5] and form a "lock-and-key" complementary structure, which is a foundation of accurate target identification of ELISA. The second principle is that enzymes catalyze color reactions with substrates to produce measurable colored products for quantitative analysis. The intensity of color is related to the amount of target molecules in the sample [3].

2.2. The types and the procedure of ELISA

According to differences in antigens, antibodies, substrates and experimental conditions, ELISA can be classified into four types: direct ELISA, indirect ELISA, sandwich ELISA and competitive ELISA [6]. Each types of ELISA must be included adding substrate to make the color reaction.

2.2.1. Direct ELISA

This method is used to detect the antibody. Immobilized the antigen on the 96-well microplate and then add enzyme-labeled primary antibody to incubate for the detection. It requires only a single incubation step, featuring a simple procedure and fewer operational steps. But it is time-consuming, associated with high cost and lack of signal amplification [5].

2.2.2. Indirect ELISA

This method is used to detect the specific antibody. The antigen is immobilized on the 96-well microplate and then add the serum with specific antibodies and bind with antigen. Next, add enzyme-labeled secondary antibody and form an antigen-antibody-enzyme-labeled secondary antibody complex for detection [7]. In this method, secondary antibodies have high universality; one enzyme-linked secondary antibody can be compatible with multiple primary antibodies, thus reducing costs.

2.2.3. Sandwich ELISA

This method is used to detect the macromolecular antigen and it is also the commonly used type of ELISA in clinical testing. The antibody is immobilized on the 96-well microplate and then add the samples with antigen and bind with antibody. Subsequently, enzyme-labeled antibodies are added to form an antibody–antigen–enzyme-labeled antibody complex for detection [7]. This has high specificity and sensitivity and suitable for complex sample like serum and body fluid.

2.2.4. Competitive ELISA

This method is used to detect the antigen or antibody. The antibody is immobilized on the 96-well microplate while the test sample and the enzyme-labeled antigen are added. The test antigen

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compete with the enzyme-labeled antigen for binding to the immobilized antibody and for detection [7]. However, signal intensity declines as the concentration of target antigens rises. Thus, it is suitable for small-molecule antigens such as hormones and toxins.

3. The clinical application of ELISA in human diseases

Nowadays, ELISA is widely used for detect some human diseases and plays an important role. It can help people detect diseases efficiently and receive timely prevention. Here are some human diseases which can use ELISA to detection.

3.1. Tumor screening and detection

ELISA is a widely used technology to screen and detect the tumor. For example, The high-throughput ELISA technology can detect the carcinoembryonic antigen (CEA). CEA belongs to oncofetal antigen and most tumors that secrete CEA are located in hollow organs, particularly in colon stomach and rectum so that the CEA severs as a useful biomarker to detect the malignant tumor of digestive tract. Automated ELISA immunoanalyzer can be applied to detect CEA, providing useful data and achieving the detection of malignant digestive tract tumors [8]. In the serological diagnosis of lung cancer, ELISA is a common detection techniques because of its high sensitive and specificity. Serum mucin 1 (MUC1) is a very important tumor marker and in the lung cancer have the high expression of MUC1 [9]. Therefore, a novel method uses double-antibody indirect sandwich ELISA to detect serum MUC1 levels. The measurement of serum MUC1 can serve as an auxiliary index to differentiate benign and malignant lung lesions [9]. ELISA is also widely used in liver cancer. For example, The double-antibody sandwich ELISA method can detect the level of midkine (MK) in the serum of patients with hepatocellular carcinoma (HCC) and based on the concentration of the MK and the diagnostic performance parameters such as sensitivity, specificity and positive rate to diagnose HCC [10].

3.2. Infectious diseases

The technology of ELISA is prevailed in the diagnosis of infectious diseases. For example, acquired immune deficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) infection. To detect the HIV, ELISA is one of the most widely used methods with double-antigen sandwich ELISA to coats the antigen on the microplate, after adding serum and washing, enzyme-labeled antigen is added. This method is easy to operate and has high accuracy [11]. The hepatitis B is caused by hepatitis B virus (HBV). The common method for detecting HBV is the indirect ELISA [12]. In the detect of hepatitis C virus (HCV), the double-antigen sandwich ELISA is the common method. However, it exhibits relatively high missed diagnosis and misdiagnosis rates, so it is only applicable to preliminary screening [13]. To increase the rate of disease screening, it can make the combination with ELISA and nucleic acid testing method [13].

3.3. Allergic diseases

Food allergy is a common immune disorder. Its pathogenesis is mainly related to the complex regulation of cluster of differentiation 4-positive T (CD4+T) cells in the immune system, including T helper type 2 (Th2) cell polarization response, imbalance of regulatory T (Treg)/T helper type 17 (Th17) cells and so on.Food allergies have become a major public health concern, with their incidence rising annually. It is one of the most prevalent allergic diseases and seriously impairs the

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quality of life of patients and their families. In Europe and America, peanuts are one of the main allergens. The sandwich ELISA is a reliable and widely used methods for detecting allergens, some researchers have previously used to detect the allergen specific immunoglobulin E (IgE) levels in serum samples from patients with allergic diseases with peanuts [14]. This method is also suitable for the detection of virus allergens, food allergens with shrimp, eggs and milk and it can also detect medicine allergens. Also, the test results of ELISA is not affected by the use of antihistamine drugs, and there is no need to conduct a skin prick test so this method is suitable for allergic patients with severe skin diseases.

4. Strengths and weakness of ELISA

4.1. Strengths of ELISA

ELISA possesses many advantages, including simplicity, convenience and rapidity, as well as high specificity and sensitivity. It can effectively improve the detection rate and remains widely applied in medical institutions and blood supply establishments at present [15, 16]. Simple: The steps are clear and standardized and do not required complex professional techniques or ripe experience. Also, the ELISA has the low required of the instrument like the automated ELISA immunoanalyzer is generally an automated operation and the operation of the constant temperature incubator and microplate reader are very simple. Convenient and rapid: the detection reagents are supplied as a pre-prepared reagent kit and do not need manual preparation. Strong specificity and high sensitivity: the antigen and antibody highly specific binding and can detect the target objects accurately [14]. In addition, ELISA is safe because there is no radioactive materials.

4.2. Weakness of ELISA

There are also some limitation of ELISA, like the dependence on the immune response mediated by antigens and antibodies is significant. Therefor, conducting blood test on on patients using ELISA is likely to result in an in,crease in false negative result [13]. Another disadvantage is that even with high levels of target analytes, abnormal signal attenuation and falsely low readings may occur [3]. Also, it is a time consuming technology, although it is faster than RIA, ELISA stills requires multiple washing steps and incubation, which limits its application in urgent testing.

4.3. The future prospect of ELISA

In the future, to make a combination with the ELISA and microfluidics and biosensors to enable the miniaturization and reduce reagents consumption. The high-throughput ELISA can realize simultaneous detection with massive samples and decrease the manual operation error and improve the detection accuracy effectively [8]. Additionally, it is a good way to make the combination with ELISA and artificial intelligent (AI). ELISA can also be integrated with laboratory information systems, which greatly facilitates experimental operations.

5. Conclusion

ELISA remains an indispensable methodology in global clinical laboratories. It can offer different methods and procedures for different diseases. This paper summarizes the main types of ELISA, including direct ELISA, indirect ELISA, sandwich ELISA and competitive ELISA, and describes their procedures systematically based on the basic principles of ELISA: the highly specific binding

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between antigens and antibodies and enzyme-catalyzed color reactions. And analyse the clinical application of ELISA in human diseases with tumor screening and detection like digestive system malignancy, lung cancer and liver cancer, infectious diseases like HIV, HBV and HCV and allergic diseases like food allergies to provide a suitable and better method in ELSIA. ELISA shows multiple prominent strengths in clinical practice including strong specificity, high sensitivity, more safe, simple convenient have higher economic benefits. However, ELISA still has certain limitations, including false results (false positives and false negatives), the requirement for high-quality antibodies and time-consuming operation. This can make some restrictions of ELISA in the clinical applications. But we can make the combination with another technology like nucleic acid testing method, PCR or microfluidics technology, this can better improve accuracy and precision rate especially in some infectious diseases. In the future, the ELISA should be continuously improved. With the integrated application of advanced technologies such as microfluidic chips, automated platforms and intelligent analysis, ELISA will develop towards the simultaneous detection, high sensitivity, high throughput development and its clinical application scope will also be expanded. This enhance the levels of clinical diagnosis and treatment and provide strong support for safeguarding human health.

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