ELISA Immunological Reagents

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7.1 ELISA Immunological Reagents Overview

Enzyme-linked immunosorbent assay (ELISA) has experienced vigorous development for nearly 50 years since it was proposed in 1971. At present, the common detection methods of ELISA immunoreagents include double antibody sandwich method and double antigen sandwich method, competition method, indirect method, capture method, and biotin-avidin ELISA [1]. Because of its simplicity, stability, ease of automated operation, and variety of detection reagents (Table 7.1), ELISA detection is widely used in laboratory departments of large and medium-sized hospitals, and it is the main technical means of blood source screening at present. Common ELISA immunodetection items mainly cover: hepatitis series, respiratory series, venereal disease series, tumor marker series, TORCH (Toxoplasma, Rubella virus, Cytomegalovirus, Herpes simplex virus, and others) series, children's digestive tract series, etc.

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Table 7.1 List of mainstream ELISA immunological reagents

Serial no.	Diagnostic category	Mainstream detection reagents	Clinical applications
1	Hepatitis series	Hepatitis A virus IgM antibody test kit	Infection detection and treatment monitoring for hepatitis A, B, C, D and E
		Hepatitis A virus IgG antibody test kit	
		Hepatitis B virus surface antigen diagnosis kit	
		Hepatitis B virus surface antibody test kit	
		Hepatitis B virus e antigen test kit	
		Hepatitis B virus e antibody test kit	
		Hepatitis B virus core antibody test kit	
		Hepatitis B virus core antibody (IgM) test kit	
		Hepatitis B virus pre-S1 antigen test kit	
		Hepatitis B virus nucleic acid associated antigen test kit	
		Hepatitis B virus surface antigen confirmation kit (neutralization test)	
		Hepatitis C virus antibody diagnosis kit	
		Hepatitis C virus core antigen test kit	
		Hepatitis D virus IgM antibody test kit	
		Hepatitis D virus IgG antibody test kit	
		Hepatitis E virus antigen test kit	
		Hepatitis E virus IgM antibody test kit	
		Hepatitis E virus IgG antibody test kit	
2	Respiratory series	Mycobacterium tuberculosis IgG antibody test kit	Auxiliary diagnosis of novel coronavirus infection, as well as screening, diagnosis and treatment monitoring of other respiratory diseases
		Tuberculosis infection T-cell test kit	
		Influenza A (H1N1) virus (2009) HA antigen test kit	
		Novel coronavirus(2019-nCoV) IgM/IgG antibody test kit	
		Novel coronavirus (2019-nCoV) IgM antibody test kit	
		Chlamydia pneumonia IgM antibody test kit	
		Chlamydia pneumonia IgG antibody test kit	
		EB virus VCA IgA antibody diagnosis kit	
3	Venereal disease series	Human immunodeficiency virus antibody diagnosis kit	Infection detection and treatment monitoring of sexually transmitted diseases
		Human immunodeficiency virus antigen antibody diagnosis kit	
		Treponema pallidum antibody diagnosis kit	

Table 7.1 (continued)

Serial no.	Diagnostic category	Mainstream detection reagents	Clinical applications
4	Tumor marker series	Alpha fetal protein test kit	Screening, diagnosis, and postoperative monitoring of common cancers
		Carcino-embryonic antigen test kit	
		Six tumor markers test kit	
		Total prostate-specific antigen test kit	
		Free prostate-specific antigen test kit	
5	TORCH series	Toxoplasma IgM antibody test kit	TORCH, pre-pregnancy, or early pregnancy examination, differential diagnosis of prenatal infection
		Toxoplasma IgG antibody test kit	
		Rubella virus IgM antibody test kit	
		Rubella virus IgG antibody test kit	
		Cytomegalovirus IgM antibody test kit	
		Cytomegalovirus IgG antibody test kit	
		Herpes simplex virus type I IgM antibody test kit	
		Herpes simplex virus type I IgG antibody test kit	
		Herpes simplex virus type II IgM antibody test kit	
		Herpes simplex virus type II IgG antibody test kit	
6	Children's digestive tract series	Coxsackie virus A16 IgM antibody test kit	Screening and diagnosis of hand-foot-mouth disease in children
		Enterovirus 71 IgM antibody test kit	
7	Others	Hantavirus IgM antibody test kit	
		Hantavirus IgG antibody test kit	
		Human rabies virus IgG antibody test kit	
		Leukocyte differentiation antigen CD8 test kit	
		Leukocyte differentiation antigen CD4 test kit	
		Talaromyces marneffei antigen test kit	
		Dengue fever virus NS1 antigen test kit	
		Dengue fever virus IgG antibody test kit	
		Nucleoprotein resistance Sp100 IgG antibody test kit	
		Varicella-zoster virus IgG antibody test kit	
		Neutrophil gelatinase-associated lipocalin test kit	
		Anti-m2 mitochondria IgG antibody test kit	
		Anti-cyclic citrullinated peptide antibody test kit	
		Anti-cyclic citrullinated peptide IgG antibody test kit	
		Encephalitis B virus IgM antibody test kit	
		Autoimmune disease ENA antibody test kit	

7.2 Introduction of ELISA Immunological Reagents from Different Manufacturers

According to the projection of the number of approvals reported by the Chinese Academy of Inspection and Quarantine, the ELISA immunological reagents are estimated to be about 1.5 billion servings in 2021, and the total number of blood screening (pharmaceuticals) is about 670 million servings, and the market capacity is estimated to be about 1.6 billion RMB. At present, the domestic market in China is basically dominated by domestic reagents, and the representative manufacturers of domestic reagents are Shanghai Kehua, Beijing WANTAI, InTec, etc. The details are as follows:

7.2.1 Shanghai Kehua Bio-Engineering Co., Ltd.

Shanghai Kehua Bio-Engineering Co., Ltd. was founded in 1981, and it was listed in the SME Board of Shenzhen Stock Exchange in 2004. The main business of the company covers enzyme-linked immunity, chemiluminescence, colloidal gold immunity, biochemistry, nucleic acid and supporting medical testing instruments, and it is the earliest company in China to launch test kits for two-half detection of hepatitis B. Kehua has rich products in hepatitis diagnosis, providing users with antigen and antibody test kits for hepatitis A, B, C, and E, and also providing test kits for HIV, syphilis, and tumor markers. The above products meet the needs of clinical and blood collection and supply systems.

7.2.2 Beijing WANTAI Biological Pharmacy Enterprise Co., Ltd.

Beijing WANTAI Biological Pharmacy Enterprise Co., Ltd. founded in 1991, is a high-tech enterprise engaged in the research, development and production of biological diagnostic reagents and vaccines, and was listed on the Shanghai stock exchange in 2020. The company's main business includes enzyme-linked immunity, chemiluminescence, colloidal gold immunity, nucleic acid, blood and supporting medical testing instruments, and its enzyme-immune products cover hepatitis, venereal diseases, respiratory diseases and digestive tract diseases, etc.

7.2.3 InTec PRODUCTS, INC

InTec PRODUCTS, INC was established in 1989, is a high-tech enterprise specializing in the research, development, production and sales of in vitro diagnostic products. The company's main business includes enzyme immunoassay, colloidal gold immunoassay and biochemistry. The enzyme immunoassay products mainly include hepatitis series and venereal disease series.

7.2.4 Shanghai Rongsheng Biotech Co., Ltd.

Shanghai Rongsheng Biotech Co., Ltd. (formerly Shanghai Rongsheng Biotechnology Co., Ltd.) was established in 1988. The company's products cover enzyme immunoassay, colloidal gold immunoassay, biochemical and vaccine, etc. The enzyme immune products mainly include hepatitis series, venereal disease series, and respiratory diseases.

7.2.5 BGI·GBI Biotech Co., Ltd.

GBI Biotechnology (Beijing) Co., Ltd. was established in 1994. In April 2003, the company officially changed its name to BGI·GBI Biotech Co., Ltd. Based on the complete series of enzyme immunoassay diagnostic reagents, the company developed nucleic acid diagnostic products and biochip products, and our enzyme immunoassay products mainly include hepatitis series, venereal diseases, and Epstein–Barr virus.

7.2.6 Beijing Kinghawk Pharmaceutical Co., Ltd.

Beijing Kinghawk Pharmaceutical Co., Ltd. established in 1993, is a high-tech enterprise specializing in biotechnology and product development. The company has six production lines of gold standard, enzyme immunoassay, fluorescence quantitative PCR, blood typing, medical equipment, and quality control products. The ELISA products mainly include hepatitis B series and venereal disease series.

7.3 ELISA Immunological Reagents Development Trend and Prospect

ELISA is a conventional detection technique with mature method, reliable technique, and simple operation. It has irreplaceable clinical application value. With the development of new techniques and methods, ELISA immunoassay system is developing rapidly toward automation and high efficiency.

7.3.1 Technology Upgrade Brings Application Expansion

Following the application of genetically engineered antibodies after monoclonal antibodies, the specificity of the assay and the accuracy of the analysis have been significantly improved, further enhancing the advantages of ELISA technology. ELISA has been developed in the continuous integration with modern technology, and through the progress of enzyme labeling technology, the application of labeled enzymes has been extended from horseradish peroxidase to alkaline phosphatase,

β-galactosidase, urease, glucose 6 phosphate dehydrogenase, glucose oxidase and more than 20 other enzymes. In addition, the solid phase carriers for ELISA applications have developed from polyethylene or polystyrene microplates to nitrocellulose membranes, activated filter paper, silica sheets, nylon, various solid phase particles synthesized using polymeric materials, etc. At present, ELISA combined with other labeled immunoassays, new assays of ELISA have been carried out, such as fluorescent enzyme immunoassay, speckle enzyme-linked immunosorbent assay, magnetic particle ELISA, and chromatography-ELISA [2]. ELISA technology has stepped out of the pharmaceutical and clinical fields into the agriculture, fishery, animal husbandry, and food processing industries.

7.3.2 Increasing Number of ELISA Projects

According to incomplete statistics, there are more than a thousand test items established or developed by ELISA technology, and as many as 300 kinds of test kits can be purchased by commercial institutions, including nearly more than 100 kinds of commonly used test kits. In summary, all the substances corresponding to antigens and antibodies can be obtained, most of which can be established by ELISA.

7.3.3 Application of Blood Collection and Supply System Laboratory

According to the National Health Commission of China, the blood donation rate in China exceeded 12‰ in 2021, and the number of blood donations exceeded 16 million. At present, ELISA reagent is the first choice of in vitro diagnostic reagent for blood screening, which is widely used in blood collection and supply system. Most blood collection and supply institutions still adopt the detection mode of two ELISA + one nucleic acid detection (NAT), and chemiluminescent immunoassay (CLIA) blood detection is still in the stage of scientific research results comparison and testing. There are hundreds of blood stations and a larger number of plasma collection stations and biological products companies in China. ELISA reagents still have market development potential from the view of several diseases with large demand and mature development.

7.3.4 Application During the Novel Coronavirus Pandemic

In the special period of novel coronavirus outbreak, based on the mature ELISA technology platform, effective novel coronavirus serological antibody detection kits, and novel coronavirus nasopharyngeal swab and throat swab antigen detection kits were developed to meet the needs of epidemic control. The combined application of nucleic acid, antigen and antibody detection methods can shorten the detection window period and improve the positive detection rate, which is conducive to the detection, diagnosis, and prevention and control of cases.

7.3.5 ELISA Reagent Detection Mode Toward Full Automation, High-Throughput Development

With the growth of ELISA test volume, quality control and biosafety awareness in medical institutions, semi-automatic enzyme immunoassay analyzer can no longer meet the current demand for laboratory automation. There are fully automated ELISA analyzers that can combine the characteristics and parameters of reagents to introduce fully automated and high-throughput solutions to achieve full automation and high throughput of ELISA reagent detection mode while reducing costs. Full automation of ELISA reagent detection mode reduces the need for operators, reduces the work intensity of operators, and improves work efficiency. In addition, the full automation of ELISA reagent detection mode has a very big advantage in detecting infectious samples, which can effectively avoid the risk of infection due to misuse or accident.

In a word, with the development of science and technological innovation, ELISA technology will become more and more perfect, the degree of automation, accuracy and precision is getting better and better, will be better to serve mankind.

Declaration Chao Chen, Yuming Ji, Jianfang Liu and Haifeng Shi are employees of Shanghai Kehua Bio-Engineering Co., Ltd.

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