Study on Enzyme-Linked Immunosorbent as an Automatic Control System

Mu Zhou[1], Lianqing Zhu*[2], Mingli Dong[2]

1.Chongqing jiao tong university, Chong Qing, China, 400074
2.Beijing Information Science & Technology University, Beijing, China, 100192

***Abstract* —The system adopted the method of the spectral detection technology to measure the enzyme-linked immunosorbent assay (ELISA/ EIA"), and measured enzyme immunoassay parameters by multi-wavelength spectrophotometry. The automatic ELISA analysis system, which was an automated flexible laboratory platform and a multi-tasked control platform, had the drawer layer mode of multichannel reader plate units and multichannel incubation units. The software of the system based on windows had the ability of parallel processing and multi-tasking and designed according to genetic simulated degradation algorithm. The system fills up the domestic technology gap of ELISA.**

Keywords - ELISA；Automatic control；Spectroscopy; Genetic simulate degradation algorithms

I. INTRODUCTION

The ELISA is a basic and conventional clinical examination detection technique in the modern medicine. The ELISA determination has become the dominant technology in the infectious serology markers (such as hepatitis, HIV, teratogenic pathogen), tumor markers, endocrine and other indicators of clinical immunology testing, because the ELISA determination had the features of simple operation and reliable technology. The reasons of clinical testing error in the laboratory, about 79%, is due to the improper manually pipetting and sample handling in the experimental process, according to the surveying report by the College of American Pathology (CAP). Therefore, the study on the automated enzyme immunoassay test system has important scientific significance and huge market demand [2, 6, 10, 11].

The automatic ELISA test system in abroad can be traced back to the end of the 1990s in the last century. The first generation of automatic ELISA analysis system is produced by the company of HAMILTON in the Switzerland and is considered to be "saving labor and little improve efficiency ". The basic technical characteristics of the second generation of automatic ELISA analysis system is the single track and is considered to be a full-automatic enzyme analysis system excluding a sampling device, or called "post-processing system". The basic characteristics of the third generation of automatic ELISA analysis system have the function of multitasking, multi-channel, and a full realization the parallel processing[1, 3, 4, 5, 7, 8].

II. SYSTEM DESIGN

The system adopted the spectral detection technology as the basic measurement method of ELISA to design a modern optical-mechanic-electronic integration automatic enzyme analysis system. The system has a module of sample reagent rack, a module of automatic samples diluted, a module of automatic sampling, a module of automatic incubation buffer, a module of automatic washing board, a module of automatic reading board, a module of enzyme mark plate automatic transmission, a module of trace reagent sampling by the syringe pump, a module of incubation temperature control, and a module of enzyme mark plate vibration. The automatic ELISA analysis system has achieved an automatic unattended processing including the identification of samples, sampling, mixing, dilution, incubation, wash the plate, read the plate and print the report, so it is a fully automated ELISA analysis. The automatic enzyme immunoassay analysis system based on the technology of spectrum is shown in figure 1[1, 9, 14].

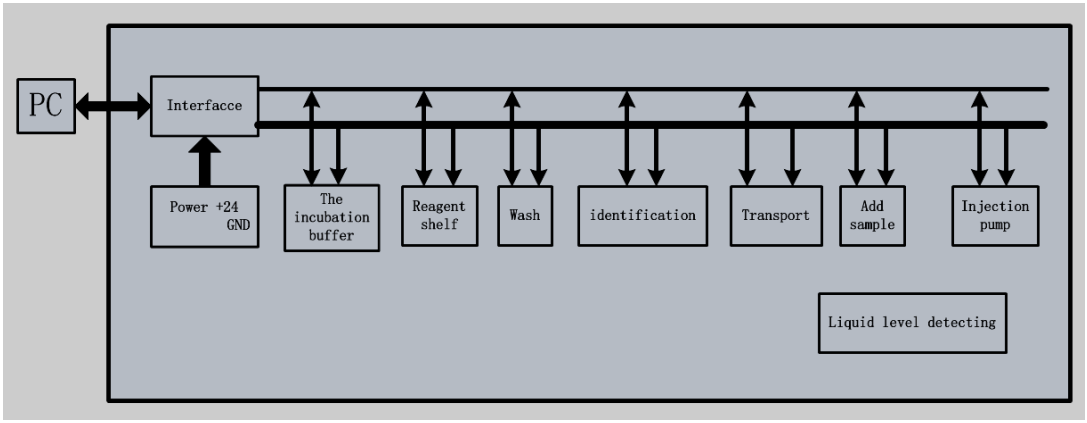

Fig.1 System function module chart

The system control diagram is shown in Fig. 2. The system comprises the control of sample movement and sampling amount, the control of transferring a sample plate, the control of the accuracy of reading plate, the control of residual quantity of washing plate, the control of incubation temperature. The system designs the control circuit module based on MSP430 and designs algorithm and program which controls the operation of each part function. The user interface and control program of the software designed with visual C++ to realize simple and reasonable operation.

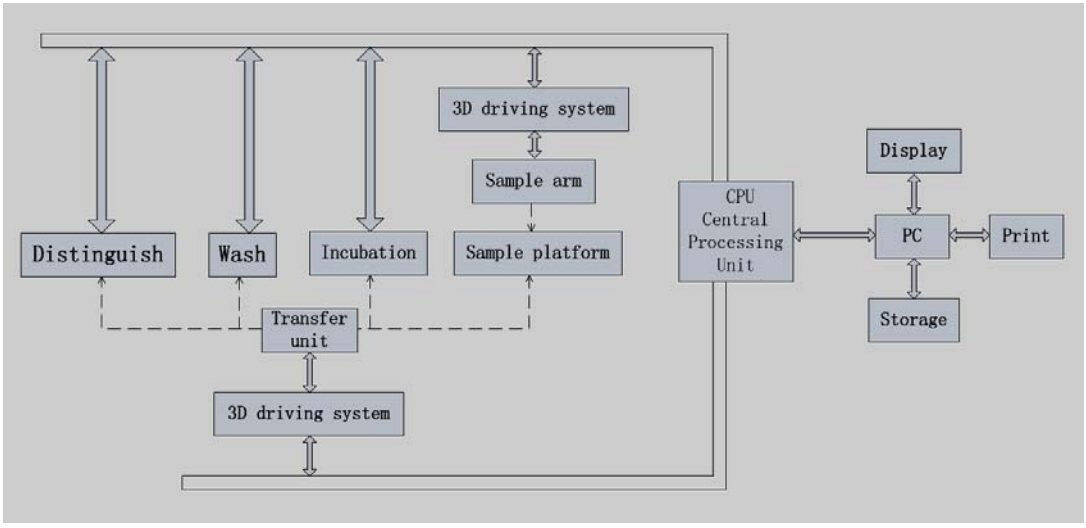

Fig.2 System control diagram

The working principle diagram of the optical system in the enzyme-linked

immunosorbent assay is shown in Fig. 3. The system composes light source, filters, optical fiber, lens and optical battery. Their selection and matching is very important.

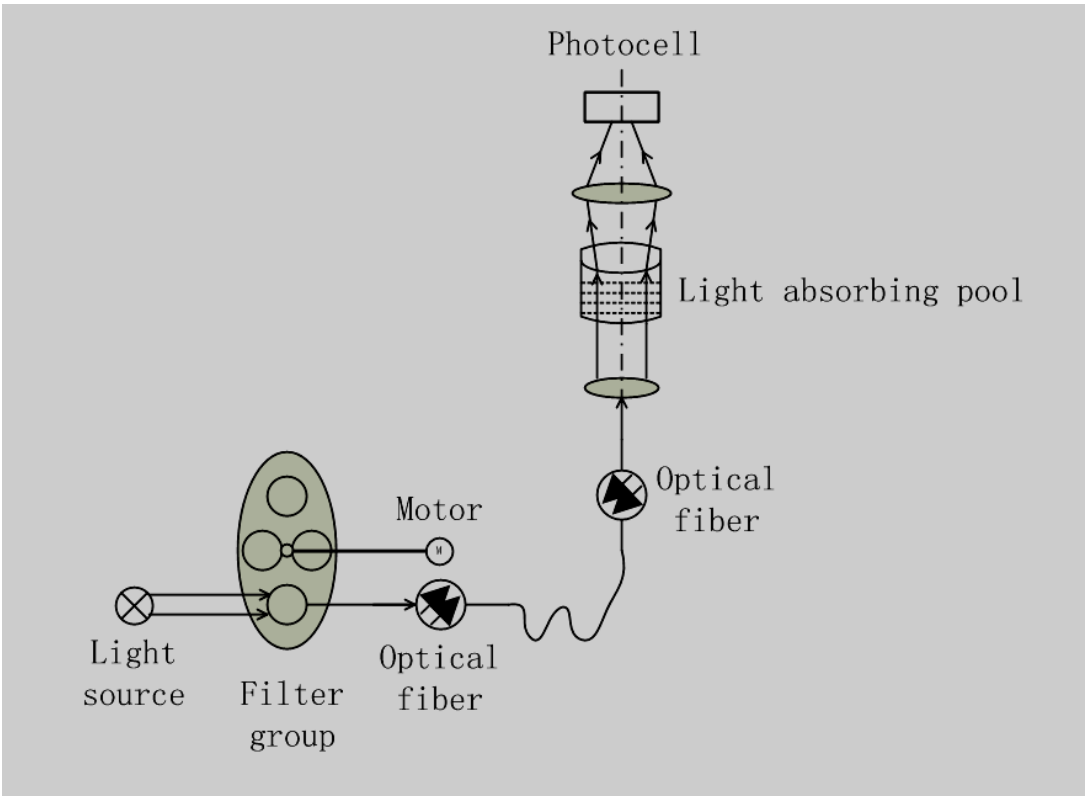

Fig.3 Optical system diagram

The light source of the system chooses halogen tungsten lamp whose wavelength is 400nm - 700nm. The system demands a relatively narrow bandwidth and a high transmittance interferential filter which is adopted to get five kinds of monochromatic light whose wavelength is 405nm, 450nm, 490nm, 570nm and 630nm, and the bandwidth must be less than 10nm under the test. In order to decrease the influence of the collection of the incident light of optical fiber and light loss in the transmission process to the measurement system, the polymethylmethacrylate (PMMA) optical fiber, that the numerical aperture is 0.6 and optical loss is less than 180db/km, is used to transmit the light in the system. The photocell must have the features that is wide spectral response range, high sensitivity, good linear and small dark current to ensure high luminous flux. It is mainly used to test light energy that wavelength is the scope from 320 nm to 1000 nm, and its response peak wavelength is 920 nm. According to the law of light absorption in the spectral analysis theory, the wavelength range of absorption spectrum of the enzyme immunoassay test samples were determined by ultraviolet (UV)-visible spectrophotometer. Several different wavelength filters can be chose by the color of the reaction liquid. The light transmittance of a solution is obtained by measuring the intensity ratio of the incident light and transmitted light. Then absorbance is equal to the negative logarithm of the light transmittance.

III. SOFTWARE DESIGN

In developed countries, the automatic enzyme analysis system is developing in the direction of the processing system integration and networked analysis system. The enzyme-linked immune experiment is very complex compared with the process of biochemical reaction analyzer experiment. That requires that the automatic enzyme analysis system should have an ability of multitask parallel processing, in particular have free task resource management system to ensure adding menu of tasks and inserting emergency at any time, and enzyme analysis system is not affected by the influence of sampling in the experiment. This means that the pretreatment must be independence from the post-treatment, so the system can be high performance and high efficiency, achieve optimization process and maximum analysis capacity. The whole system composes a computer, a software and a mechanical structure[12, 13].

The system application software controls the processing

of test and communication with the control circuit by serial port. The system software compiled in the Visual Studio C++ 6.0 integrated development environment. It provides primarily a simple, friendly, and humanized man-machine interface. The interface is shown in Fig.4.

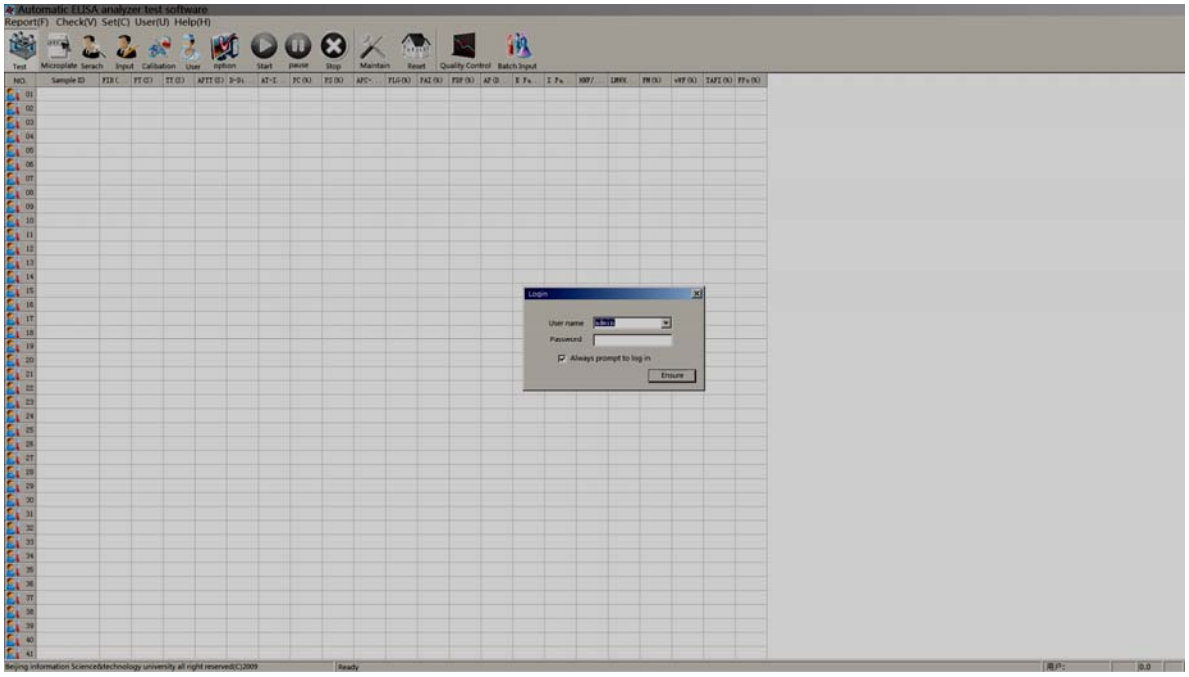

Fig.4 The interface of the application software

The communication format is shown in table I. The serial communication adopts RS 485 broadcasting master-slave communication mode. The slave computer waits an instruction from host computer and replies it, at the same time it can not actively send a message to any other computers in any time. All the communication data and instruction are the ASCII of hex. The valid data character is 0 to 9 and A to F in the capital form. The system uses the CRC(cyclic redundancy check) which is widely used in the field of measurement and communications. The system achieves the CRC by the software algorithm in the case of without the hardware support. The CRC polynomial uses CRC16. First, a binary sequences data left shifts 16 bit, then the result divided by a polynomial, the remainder is the CRC16 code. The algorithm has the half querying method, the whole querying method, direct calculation method and so on. The half querying method is moderate in the calculation speed and memory requirements, so it is suitable for data check in the 8 bit single chip.

TABLE I. COMMUNICATION TRANSMISSION FRAME FORMAT

Title	Start symbol	Slave computer address	Instruction	Data	CRC	End symbol
Number(byte)	1	1	1	N	4	1

IV. CONCLUSION

The automate enzyme immune analysis system based on the technology of spectrum, broke foreign monopoly, and greatly reduced the cost of enzyme-linked immunosorbent assay. Secondly, it developed the technology of photodetector in the biomedical field, and integrated the different subject include the life sciences, the biomedical testing, information technology, automation technology and computer control technology and so on, laid the foundation of the full automated laboratory. The ELISA analysis technology achieved the automated detection technology, the commercial reagents, the standardized detection methods, the trace samples, the normative quality supervision. It promotes technological progress in the enzyme-linked immunosorbent assays in the biomedical field, changes the situation of without the high product in the analytical techniques and testing equipment. It

improves the level of automation of biomedical testing equipment and provides technical support to promote the flow of knowledge and technology transfer, promotes the improvement of high-tech talent and innovation ability. The system composes optics, electronics, precision machinery and computer control technology, and is a typical optical-electromechanical integration testing system, which promotes the application and promotion in Beijing optical-electromechanical integration and information technology. At the same time driving development of the machining of the upstream machinery, electronic product and other industries, it is of great significance to promote socio-economic development in Beijing.

The modern automated enzyme immunoassay system based on the technology of spectral detection is a system of the optical-mechanical electrical integration, measures enzyme immunoassay reaction parameters by multi-wavelength spectrophotometric measurement. The system completes the sample reagent pipetting, sample dilution, incubation buffer of enzymatic colored product, cleaning test board, the measurements of enzymatic colored composition and absorbance, transferring enzyme standard board, and other trace reagent sampling modules with injection pump. Its core is msp430, communicates with the PC by USB, and completes the signal acquisition, amplification and operations through drivers program, communication protocols and upper layer control software.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGMENT

This work is support by the key project of Beijing natural science foundation (NO.KZ201010772032), the projects of Beijing engineering research center of photoelectric information and instrument (NO.GD20110011, NO. GD2011006)

REFERENCES

[1] Farahmand, E., Ibrahim, F., Hosseini, S., Rothan, H.A., Mokhtar, M.S., Djordjevic, I., "Optimization of ELISA buffers volume for CD-based microfluidic biosensors," Biomedical Engineering and Sciences (IECBES), 575 – 577 ,2014.

[2] Ibrahim, F., Nozari, A.A., Jahanshahi, P., Soin, N., Rahman, N.A., Dawal, S.Z.M., Kahar, M.K.B.A., Samra, K.A., Madou, M., "Analysis and experiment of centrifugal force for microfluidic ELISA CD platform", Biomedical Engineering and Sciences, 466 - 470, 2010.

[3] McGeough, C.M., O'Driscoll, S., "Camera Phone-Based Quantitative Analysis of C-Reactive Protein ELISA", Biomedical Circuits and Systems,7: 655 – 659, 2013.

[4] Takehara, H., Miyazawa, K., Noda, T., et al., "A CMOS image sensor with low fixed pattern noise suitable for lensless observation system of digital enzyme-linked immunosorbent assay", Future of Electron Devices, 32 – 33, 2013.

[5] Wen Wai, Kuan-Ju Tseng, Ju-Nan Kuo, "DMS microlens fabricated for compact disk enzyme-linked immunosorbent assay", applications, Nano/Micro Engineered and Molecular Systems (NEMS): 182 – 185, 2010.

[6] Baro de la Fuente, J.A., Perez Garcia, M.A., Gonzalez Cueli, J.C., Cifuentes, D., "A new low-cost reader system for ELISA plates based on automated analysis of digital pictures", Instrumentation and Measurement Technology Conference, 1792 - 1794, 2006.

[7] Pei-Lan Shao, Po-Ren Hsueh, Luan-Yin Chang, et al. "Development of immunoglobulin G enzyme-linked immunosorbent assay for the serodiagnosis of severe acute respiratory syndrome[J]." Journal of Biomedical Science, 12:59–64, 2005.

[8] Todd Schraw, Sidney Whiteheart. "The development of a quantitative enzyme-linked immunosorbent assay to detect human platelet factor 4[J]". TRANSFUSION, 45(5):717-724, 2005.

[9] Conrad P. Quinn, Vera A. Semenova, Cheryl M. Elie. "Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen[J]". Emerging Infectious Diseases, 10:1103-1110, 2002.

[10] Chi-Jung Huang, Jaan-Yeh Jeng, Chia-Long Lee, et al. "An Efficient Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) Detects Adenomatous Polyposis Coli (APC) Protein from Human Stool[J]". Fu-Jen Journal of Medicine, 2(3):225-231, 2004.

[11] P. Duncanson, D.R.A. Wareing, O. Jones. "Application of an automate immunomagnetic separation-enzyme immunoassay for the detection of Salmonella spp during an outbreak associated with a retail premises[J]". Letters in Applied Microbiology, 37(2):144–148, 2003.

[12] Zhao qiang Wang. "Development and Status of technology automatic ELISA analysis system". Modern testing technology and equipment.11:6-9,2003.

[13] Ning Li. "The determination Key of affected enzyme-linked immunosorbent assay", Laboratory Medicine and Clinical, 3(7), 314-315, 2006.

[14] Zhongquan Pan,zhuqing Li，Guoming Zhang，etc."The manufacture of the absorbance of the standard ELISA analyzer substances"， Measurement Technology，4：39，2005.