

# Application of Enzyme-Linked Immunosorbent Assay in Early Diagnosis of Cancer

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**Abstract.** As far as the current development of the medical community is concerned, early detection is the most effective way to deal with cancer, while enzyme-linked immunosorbent assay (ELISA) has been introduced in several diagnosis area of cancer for a long time. This research summarizes the usage of ELISA in various sections from the process of cancer's early detection and highlights its function in the process of cancer's early detection. There are several types of ELISA, this research focuses on indirect ELISA and sandwich ELISA, representing their special abilities that assist to complete the process of diagnosis of cancer. ELISA could be applied in both the detection of biomarkers and the antigen from the tumor cells, thereby helping to easier detect the existence of cancer. Moreover, ELISA is functional in data provision, which assists the construction of graphics as well as later treatments. Since ELISA could be applied in almost every section of the early detection of cancer, the development scope of its specific performance is extremely broad. Even though several problems constantly occur, the application scope of ELISA is still steadily expanding throughout the biomedical area.

**Keywords:** ELISA; cancer; biomarker.

## 1. Introduction

Up to now, people don't have an ideal understanding of cancer. Generally speaking, cancer is a combination of more than hundreds of related diseases of malignant tumor, which can be found in almost every organ or tissue from animals and plants. Cancer is caused by carcinogenesis, and carcinogenesis is an extremely diversified, complicated process that impacts hundreds of genes and gene products inside the various organisms, which are the vital substance in the regulation of a multitude of cellular functions. Once cancer is occurred inside human's body, its destruction of the human immune system will cause devastating blows to the human body. Therefore, cancer is a tremendous threat for human beings. Yet in the past few years, infection and deaths caused by cancer have been increased [1]. In 2015, a survey made by The World Health Organization (WHO) estimated that during the past few decades, for almost all countries over the world, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries. In order to prevent exposure and disruption of employment from the corona-virus disease 2019 (COVID-19) pandemic that occurred recently, numerous health care settings were closed. Thus, the diagnosis and treatment of cancer has been delayed.

Due to the fact that cancer is difficult to cure in its middle and late stages, it is better for people to discover a proper way to prevent or early detect the cancer. However, prevent cancer by adjusting people's life behavior such as give up smoking and keep a healthy diet is quite ineffective. Obviously using some devices to test and gauging cancer at its early stage is the most effective way to deal with cancer. There are multiple methods for detecting cancer, and with the advancement of time, the technology of detection of cancer is becoming more advanced. There is using artificial intelligence's specific ability to minimize the difficulty of detect the tumors in the human body since AI has advantage in aggregating multiple images as well as quantify information from images that is not detectable by the humans. Besides, people introduce biomarkers. Biomarkers can enable researchers to have better strategy in the evaluation of the disease a better evaluate and estimate the agents' effects from tissue to molecular, thereby giving new conception for clinical endpoints. Although the progress of this field has been obstructed currently because of the defect from biomarker itself (Lack the ability of validated, prevention-oriented, practical), as well as to FDA approval of molecular preventive

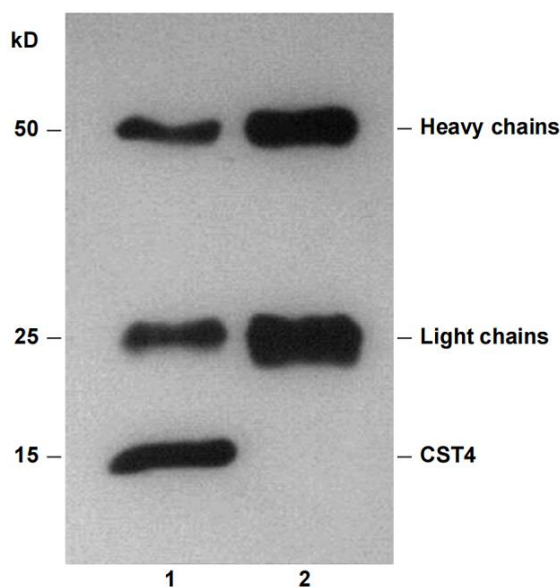
agents. The biomarkers are still popular since they are effective for testing both cancer intermediate and risk, the permission of novel molecular preventive agents and the future development of cancer test could be extensively enhanced by the biomarker's preventive response.

Enzyme linked immunosorbent assay (ELISA) is a special method that is carried out inside the animals' blood to take measurement and detection of various substance, such as hormones, antibodies, proteins and peptides. Frequently-used types of ELISA are indirect ELISA, sandwich ELISA and competitive ELISA. And because of their advantage of accurate diagnosis, highly responsive and extremely allergens in the food industry to determine the concentration of serum antibody in a virus test, the ELISA has always been involved. Therefore, ELISA is also an effective technique for the detection of cancers. For example, by calculating the concentration of special substance or modeling various curves. Just due to the fact that ELISA has been widely used in cancer detection and has shown great advantages, this research will analyze the role of ELISA in common cancers such as lung cancer and gastric cancer and draw relevant conclusions.

## 2. Application of ELISA for cancer detection

### 2.1. Blood biomarkers-based ELISA analysis

Due to the fact that two disulfide bonds in unique shape are at the C terminus of the CST4 while its molecular weight is 15 kD approximately, when extracellular matrix is hydrolyzed, CST4 would specifically combining with cysteine protease to regulate its (cysteine protease) activity, thereby inhibiting hydrolysis. Because CST4 has characteristics such as high sensitivity and low molecular weight which are involved in other biomarkers, Dou et al. had the conjecture that CST4 is a biomarker in gastrointestinal cancer [2]. In order to prepare a blood test for CST4, an analytical system based on antibody-sandwich ELISA was developed. This kind of ELISA was also preliminarily verified CST4's clinical application in the diagnosis of gastrointestinal cancer.



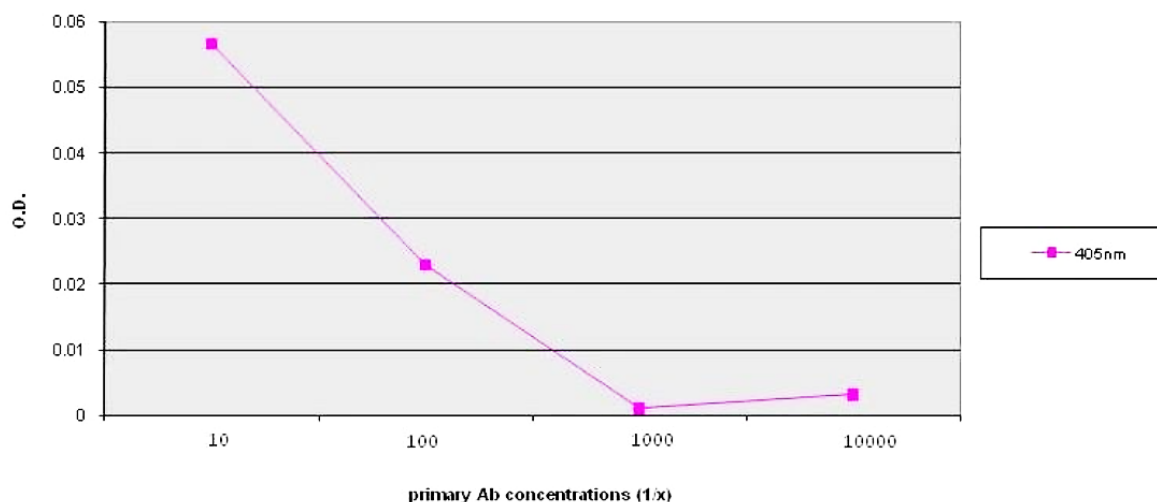
**Fig 1.** Western blotting of immunoprecipitates by detection antibody 5E4G5 [2].

When in the step of choosing the paired antibodies for CST4, after analyzing the epitopes of antibodies, they (5D2F2 and 5E4G5) need to be verified and native forms need to be clearly recognized, thereby sandwich ELISA was again be introduced. It's worth mentioning that they chose citrate-buffered saline as coating buffer so that the sensitivity of ELISA could be controlled in a higher level. Three lines from the antibody's heavy and light chains as well as the positive bands for CST4 were shown on the western blotting results (Fig. 1). This represents that in extracorporeal blood, the CST4 in its natural conformation could be recognizing by the capture antibody of 5D2F2. Looking through the overall detection parameters of the CST4-ELISA system, by providing with meticulous

data, it undoubtedly makes a significant contribution to the subsequent research of CST4. Besides, in the following clinical verification of antibody-sandwich ELISA analysis system of CST4, the specificity exhibited from the result is completely compliance with industry standard, which again testified that ELISA has a perfect accuracy.

## 2.2. Antibody-based ELISA analysis

Mycoplasmas (class Mollicutes) are a kind of prokaryotic, which has the trait of less organelle, wall-free and tiny that can assist it to choose the intracellular fluid of cell and even the cell membrane of eukaryotic cell for its residence. They are the smallest organisms (approximately 0.25  $\mu\text{m}$ ) that have been discovered to be able to self-replicate, with genomes of about 580-1200 kbp [3]. Several human pathogens have been well proved that are induced by mycoplasmas, and it is important for people to have a better understanding of the relation between human disease and mycoplasmas. In this case, from the localized prostate cancer that was diagnosis in 2011 [4]. Using tumor cells extracts, after intralymphatically immunized several early stage cancer patients, Fared et al. calculated and analyzed their immune response [5]. However, for those patients whose tumors failed to regress, some antigens were not detected due to their similar tiny shape. Taking a mycoplasmal protein for example, it was extracted from *M. hyorhinis*, called the 38-kDa. This kind of protein was particularly designated. In order to fight against *M. hyorhinismale*, serum antibodies (IgG and IgM) could be manufactured inside male's body, and Urbanek et al. developed an indirect ELISA for the detection [4].



**Fig 2.** Detection limits analysis by using the developed ELISA [4].

Besides the normal reagent operations, it is worth mentioning that the disaccharide, Gal1a1,3 Gal was chosen as the internal control [4]. As for the reasons, Gal1a1,3 Gal is presented in almost all human beings, and for every normal human individual, IgG antibodies could be found in their high titers of the serum, while all of them are constantly produced throughout life because of natural metabolism. There is no response to BSA alone for all the sera that tested, meanwhile an apparently positive response against Gal1a1,3 Gal-BSA is shown for all of them. Urbanek et al. determined a diagnostic test to make a comparison between positive and negative result and at the same time enabled to assure the save of the test's sensitivity and specificity maximum [4]. The duplication of all the sera samples were tested by indirect ELISA, where it can manage to make a discrimination for positive indirect ELISA assay by adding a special cutoff value [4]. Finally, the result illustrated that this novel indirect ELISA assay got high accuracy towards its aimed antibodies (Fig. 2).

## 2.3. Establishing mathematical models

When the pancreatic cancer is approaching its late stage, another disease called pancreatic ductal adenocarcinoma (PDAC) would be detected which gives patient a dismal five-year survival.

Therefore, the opportunity and significantly increase cure rates are particularly depend on early detection. Some advanced method such as blood-based biomarkers is a wise choice. However, based on recent experimental determination, which analyzed patterns of chromosomal alterations to give the method of using biomarkers enormous challenge. The research proposed that the tumor evolution may happen multiple times in a sudden that following a model of evolution called punctuated equilibrium model instead of always being sequential and gradual. In order to eliminate this dilemma, basing on punctuated equilibrium model and nature's gradual evolution, mathematical modeling was chosen by Alex Root to have a better understanding for early detection of PDAC [6]. In the coming decades, additional insights for the early detection of PDAC might be provided by the application of mathematical modeling, which could even make it possible to complete an appropriate PDAC early detection that has challenged scientists for decades. As for the data processing and further development of mathematical modeling, the citation of ELISA is essential.

When creating a one-compartment biomarker model for PDAC, following development of the tumor growth models, literature curation and estimation are involved to the parameterization of several remaining parameters for the one-compartment model. In these steps, ELISA was required to calculate the data between the new model and those borrowed from prostate or ovarian cancer models in order to minimize the great uncertainty. In another example of analyzing the sensitivity of model parameters' sensitivity when patients just got the cancer that in the earliest stage, results of various data such as volume of primary tumor given by ELISA were contributed to the subsequent image processing. Generally speaking, ELISA again showed its strategy for early detection by providing basic data for modeling charts.

#### **2.4. Screening for new biomarkers**

ELISA not only can assist biomarkers in detecting relevant antigens, but also can detect biomarkers themselves from a certain perspective to find a new biomarker. Currently, the global scientific research and medical community is still plagued by the colorectal cancer, while the detection of tumor-associated markers is the basis of diagnostic blood tests. However, in the area of cancer screening for large population, the usage of these markers has been greatly reduced on account of their lack of techniques which can improve the fault tolerance. Therefore, the importance of discovering highly specific biomarkers was highlighted. Since cancer antigens could be represented by the majority of the tumor markers, it is necessary to find new cancer biomarkers or better screening methods for improving the diagnostics of the disease. Compared to ELISA detecting the tumor antigen, higher sensitivity and specificity have been shown for auto-antibody ELISA in some of the tumor markers, while there is increasing evidence that the production of antibodies is the key for cancer patient's immune system responds to tumor antigens. Therefore, tumor markers could be indirectly quantified by those specifically developed auto-antibody detecting ELISA.

Considering reproducibility and rapidity, compared with antigen-determining kits, the auto-antibody ELISA is a better method which is really cost-saving and has a high fault tolerance. In this example, ecPKA and NNMT have been chosen as candidates for tumor markers. The cAMP-dependent PKA follows its normal function which allows it to located strictly intracellular in natural mammalian cells. For those various kinds of cancerous cells, PKA has been shown to be found in different location of the cell. The assumption that the excretion of ecPKA might elicit the induction of serum auto-antibodies against ecPKA was made, later experiments implied that the behavior of cancer antigen is discovered on ecPKA, then a diagnostic marker could be chosen from such auto-antibodies. That's why a novel ELISA method devised specially against ecPKA that based on IgG auto-antibodies have been developed [7]. Of course, there are still some problems remain. For example, due to the fact that less monoclonal antibodies on the market, using polyclonal antibodies makes the specificity of ELISA drop slightly.

## 2.5. Other cancer detection

There is another function of ELISA for confirming the feasibility of cancer detection. Back to the 1990's, combining the additional information provided by urinary cytology or the cystoscopy are common for the bladder carcinoma's monitoring and diagnosis. There is no doubt that these two methods have showed their great utility. However, sensitivity is still a significant issue that require occurred. Therefore, the choice of alternative methods that have the specificity of noninvasive and more objective is more concentrate on how to improve the detection of unknown bladder carcinoma clinically as well as how to effectively extended the intercystoscopy periods from patients who had bladder carcinoma. These conditions enrich the selection of methods, effective ways like various soluble and cellular biomarkers rapidly flood in. Based on the numerous calculations from urinary bladder carcinoma (UBC) antigen, which could be extracted in two kinds of cytokeratins from their urinary fragments, a new kind of ELISA has been invented in order to have a better command of reckon bladder carcinoma. Based on the high sensitivity and specificity of this new ELISA method, after meticulous comparison and analysis to the result, Sánchez-Carbayo et al. proved that this method of detecting tumor cells inside the voided urine samples was definitely reliable [8].

The one-step sandwich ELISA was applied for the urinary bladder carcinoma antigen. Speaking roughly, due to the fact that the sandwich ELISA principle is still holds in the UBC antigen test, the intensity of color developed at a specific numerical value was proportional to the concentrations of UBC antigens. A calculation of the levels of urinary bladder carcinoma antigen was done after plotting a standard curve, while it was also determined by the calibrators' multiple concurrent test and the information of urine samples from each microtiter plate.

After analyzing all the cystoscopy samples, the number of phenomena that related to the appearance or the disappearance of bladder carcinoma could be confirmed, then for those patients with bladder carcinoma, the mean values obtained for the various parameter from their bodies were measured and analyzed. Selected from Roc curve to calculate the cutoff values, the result from ELISA test and the same specificity of flow cytometric-derived parameters were able to make a comparison [8]. The selected cutoffs of the diagnostic characteristics from each parameter reflected the specificity of the relevant cells that made the analysis much easier.

## 2.6. Prognostic analysis of cancer

Despite early detection, ELISA can be used for the prognosis of cancer as well. Back to gastric cancer, considering its causes, since 1994, *Helicobacter pylori* (*H. pylori*) has been proved to be a great causation to cancer and was reckoned as Group I carcinogen by the International Agency for Research on Cancer. Therefore, the risk of having a gastric cancer could be reduced by the eradication of *H. pylori*. Several epidemiological studies have suggested that the prognosis of gastric cancer was related to the infection of *H. pylori* [9]. By comparing with relevant investigation, in order to summarize the relevance that the survival rate of gastric cancer with *H. pylori* status, Jia et al. performed a study that involved various methods, including ELISA [10].

In this case, *H. pylori* had unique antibodies (IgG) whose thersurgery levels of serum could be evaluated by the ELISA. Several aspects in this research had introduced ELISA not only to simplify the process of the study, but also to improve the accuracy. For example, at the beginning of the research, ELISA was used for the summary of the number that showed the positive patients (who have infected by the *H. pylori*) and negative patients in numerous variables. Thereby the research group could exclude the influence of irrelevant variables to further raise precision [10]. In order to carry on further stratified analyses that could pick out the factors that found during the experiments and check the stability of the overall estimation, the result of ELISA test proved that if retrieval method is used for stratification, using the relevant diction related to *H. pylori* for retrieval would fail to find information in some parts of the research, while can only be obtained by doing a full texts review. Compared with other studies that adopted methods which retrieved by using the terms of *H. pylori* itself, this approach is much less effective.

### 3. Conclusions

Utilizing the specificity of ELISA, from the provision of ordinary data to the confirmation of specific location of tumor cells for subsequent experiments, ELISA could be able to benefit the whole procedure of the early detection of cancer. Due to the fact that ELISA involves the advantage of simply standardization, its application prospect is continuous optimistic. Taking biomarkers for example, not only their detection targets but also themselves could be leaded by ELISA. With the continuous advancement of technology in the future, some drawbacks and limitations such as the lack of monoclonal antibody. Therefore, it can look forward to the application of ELISA technology in a wider range of fields, while ELISA continuing to play a key role in scientists' explorations and promote the continuous development of the field of life sciences.

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