

The utilization of ELISA in early detection of cancer

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Abstract. The early detection of cancer is crucial in improving treatment success rates and survival rates. The primary focus of this research is the application of enzyme-linked immunosorbent assay (ELISA) in early cancer detection, as well as the measurement methods of cancer biomarkers. It can provide an overview of the currently used cancer biomarkers and their application in ELISA. Furthermore, this research will utilize the ELISA technique to determine whether a specific substance can serve as a cancer biomarker, with a particular emphasis on its application in early cancer detection. Through a comprehensive analysis of relevant research, this research elucidates the significance of ELISA in the measurement of cancer biomarkers and its potential as a robust cancer screening tool. Finally, this research will highlight the future directions of research, focusing on the implementation of ELISA technology in detecting and identifying a wider range of cancer biomarkers for achieving more accurate early cancer diagnosis.

Keywords: ELISA; Cancer; Detection.

1. Introduction

One of the major health problems around the world is cancer, which causes millions of people dying every year. Although medical professionals have made great process in treating and managing the disease, being able to detect and identify cancer is a more important and effective method to increase the treatment rate and survival rate for patients, reduce the burden of patients' treatment and lower the cost of medical system.

Enzyme-linked immunosorbent assay (ELISA) technique, is a common method used to detect and quantify specific molecules in biological samples. The ELISA technique is a biological analysis method that is commonly used in the medical field. This technique is widely used in the field of biological research and health studies due to its high sensitivity, specificity, and great reproducibility, and that's the reason why ELISA is one of the most promising methods in the early detection of cancer, it has the ability to detect the low concentration of cancer biomarkers within the body fluid and analyze the presence and progression of cancer by measuring their quantitative level.

A cancer biomarker is a series of molecules that show abnormal expression during the development of cancer such as CA 125, PSA, AFP. These biomarkers have specificity toward a certain kind of cancer, which has great effect in the early detection of cancers. The ELISA technique is able to measure the quantity of these biomarkers and determine whether the patient has cancer. While the ELISA technique can really improve the detection of cancer, it still has some challenges such as sample processing, the choose of biomarkers and data analysis. Other than that, the ELISA is also facing the chances of combining with other molecule detection methods such as NGS and mass spectrometry, which will be helpful for improving the accuracy and predictivity and promote the early detection of cancer. This research will mainly analyze the application performance of ELISA in cancer detection.

2. The fundamental principle of ELISA

There are two commonly employed methods for ELISA measurement, but he foundation of ELISA lies in the immobilization of the antigen or antibody on a solid phase, coupled with the enzymatic labelling of the antigen or antibody. The antigens and antibodies attached to the solid support



preserved their immunological activity, while the antigens and antibodies labelled by enzyme retained both their immunological activity and the activity of enzyme [1].

One approach is employing the indirect method to assess the presence of antibodies. To determine the composition of the sample, certain composition of the sample would interact with the antigens attached to the solid support. After cleansing, the antigen-antibody complexes would be separated from other compositions of the sample. Subsequently, the enzyme labelled antibodies can be added and would also react with the antigens left on the solid surface. And now the concentration of the enzyme labelled antibody attached to the surface is proportionate to the quantity of the target antibody in the specimen, after engaging in enzymatic reactions with their corresponding substrates, the substrate shall undergo catalysis, resulting in the formation of pigmented production, and the quantity of the production would be directly related to that of the target antibody in the original sample. Therefore, it is completely feasible to conduct qualitative or quantitative Analysis according to the varying intensity of coloration [1].

The second approach is double antibody sandwich method, which is usually used to detect the presence of antigens. First the antibody needs to be incubated on the ELISA plate, followed by the addition of the sample, the antigen in the sample will react with the antibody attached to the plate to form an antigen-antibody complex after washing the impurities off, detection antibody labelled with enzymes will be added, forming an antibody-antigen-antibody complex, at last the corresponding substrates will be added and undergo catalysis [2]. The amount of corresponding substrates degradation is positively related to that of the antigens in the sample. Allowing for the determination of antigen quantity through a standard curve analysis.

3. Application of ELISA for detection of REG γ

Cancer biomarkers are substances found within cancer cells or produced abnormally due to the existence of cancer cells. It includes DNA, RNA and proteins. Usually exist in patients' tissue, body fluids, and excretions. There exists a diverse array of cancer biomarkers, with each type of cancer potentially having one or multiple corresponding biomarkers [2], which is why finding the right biomarkers is a crucial part in the detection of cancer. The qualitative and quantitative analysis of cancer biomarkers can greatly assist in diagnosing the condition of cancer, analyzing the disease, providing therapeutic guidance, monitoring future tendency of the disease.

Many researches show that the quantity of REG γ in patients with colorectal cancer were significantly increased [3]. These research shows that the REG γ -proteasome degradation system is very important for regulating a diverse range of essential biological processes by breaking down different functional protein substrates. These processes include the formation of tumor and autoimmune disorders [4], which highlights the potential of REG γ as a biomarker for colorectal cancer, inspiring researchers to explore its potential as a biomarker for colon and rectal cancer.

The researchers immobilize monoclonal antibodies on the enzyme-linked immunosorbent assay plate, followed by washing and blocking steps. Subsequently, the antigen samples are incubated and further incubated with detection antibodies and HRP conjugate. Subsequent washes are conducted, and then TMB substrate was added for color development. Finally, the reaction is terminated using sulfuric acid, and the results are analyzed by measuring the optical density (OD) value to determine the limit of detection concentration. In order to assess the sensitivity of the REG γ assay kit, researchers devised a series of antigen concentration gradients with increments of 400, 200, 100, 50, 25, 12, 6, 3, 1.5, and 0.75 ng/ml [2]. Using the aforementioned approach, they measured the changes in absorbance values at different antigen concentrations, the outcome is illustrated as depicted in Figure 1.

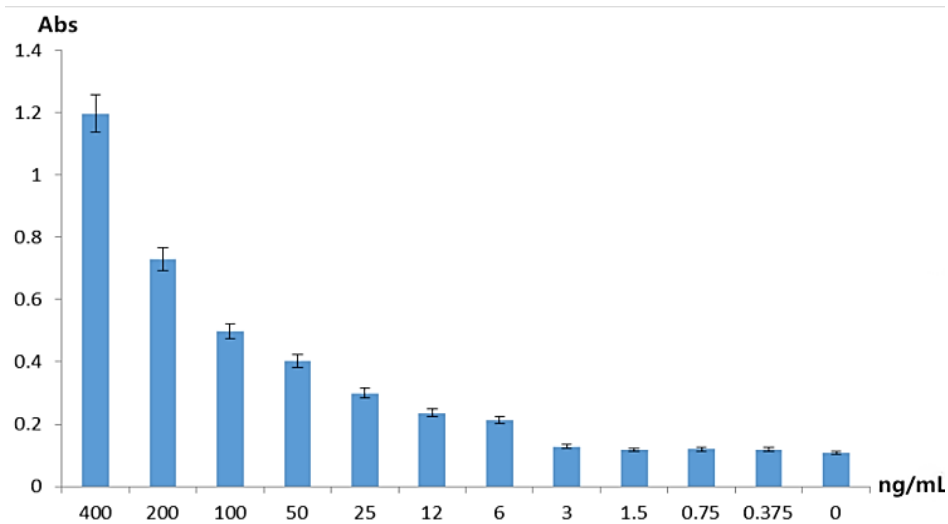


Figure 1. The changes in absorbance (Abs) values at different antigen concentrations [2].

Based on Figure 1, there appears to be a certain correlation between the magnitude of absorbance and the variation in antigen concentration when it ranges from 400 ng/ml to 3 ng/ml. However, when the antigen concentration drops below 3 ng/ml, the absorbance values remain relatively unchanged despite the gradual decrease in antigen concentration, the approximate magnitude of sensitivity is around 3 ng/ml.

4. Establishment of double-antibody sandwich ELISA to detect endosialin/TEM 1(CD248)

Research conducted both domestically and internationally has consistently shown that CD248 is highly expressed in various types of malignancies. These include sarcomas [5], skin cancer [6], and breast cancer [7]. The investigators employed recombinant CD248 antigens to immunize animals, producing both mouse polyclonal antibodies and rabbit polyclonal antibodies. These antibodies were then utilized in the development of a CD248 sandwich ELISA assay kit, aiming to contribute to the research of early diagnosis and screening for various cancers, sarcomas, and certain inflammatory diseases.

The researchers first evaluated the sensitivity of MBP-CD248 protein and GST-CD248 protein. They diluted the MBP-CD248 protein in sample dilution solution, resulting in 22 different dilutions ranging from 1200 µg/ml to 0.57 ng/ml. Similarly, they performed 20 different dilutions on the GST-CD248 protein using the same sample dilution solution. An ELISA detection method was employed using a negative control comprised of BSA protein samples with equal concentration. By analyzing the OD values and P/N ratio (average), and employing a threshold of an OD value above 0.1 and a P/N ratio ≥ 2.1 , they established the minimum detectable concentration for the assay [8]. The researchers utilized the CD248 sandwich ELISA method to detect the culture supernatant of human osteosarcoma cells. Initially, the osteosarcoma cells were reconstituted and transferred to larger culture flasks for growth. Once the cell culture reached 80%-90% density, the supernatant was collected after 108 hours of cultivation and subjected to further processing. Following centrifugation, the supernatant was collected as the sample for subsequent sandwich ELISA detection [8]. A series of diluted concentrations of MBP-CD248 protein and GST-CD248 protein, with known concentrations, were separately detected using the ELISA detection conditions established in this experiment. The results revealed a minimum detectable concentration of 18.31 ng/ml for MBP-CD248 protein and 585.94 ng/ml for GST-CD248 protein, which means that the detection sensitivity of MBP-CD248 is significantly higher than that of GST-CD248 [8].

The cell culture supernatant obtained from SJS-1 cells after 108 hours of cultivation was subjected to detection using the sandwich ELISA method established with CD248 molecular bispecific antibodies. A sample well with an OD value/blank control well OD value > 2.1 was considered positive according to the judging criteria. The experimental results confirmed the capability of the

established ELISA method to detect the CD248 molecule in the SJSA-1 cell culture supernatant [8], as shown in Figure 2.

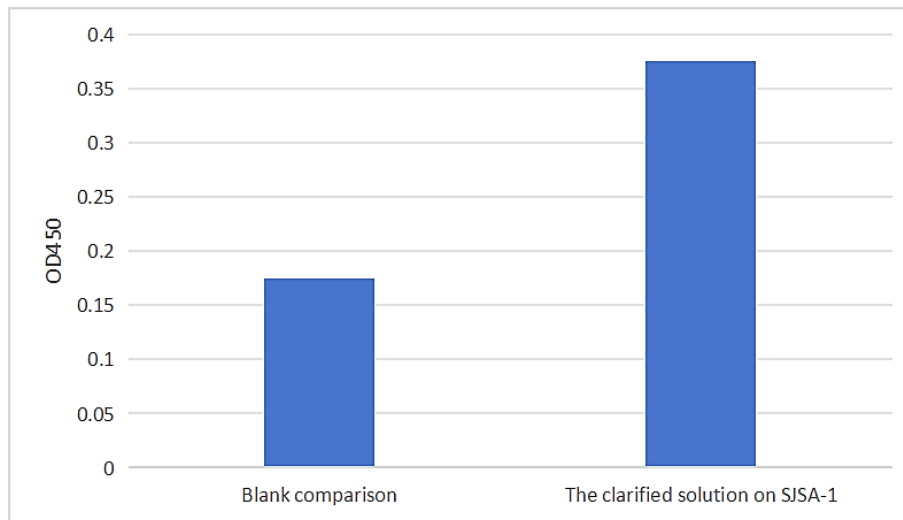


Figure 2. The result of detecting SJSA-1 culture supernatant with ELISA [8].

5. Detection of MUC1 protein in peripheral blood of adenocarcinoma patients by indirect sandwich ELISA

As a prominent macromolecule, mucin proteins exhibit exceptional properties in enveloping, lubricating, and safeguarding the luminal surfaces of various bodily glands. Notably, a plethora of transmembrane mucin proteins have been identified, among which several are believed to exert regulatory roles in tumor cells, effectively influencing their phenotypic characteristics [9]. Currently, MUC1 is extensively investigated as one of the tumor markers for adenocarcinoma diagnosis. However, the existing detection methods possess a relatively low sensitivity. In order to enhance the sensitivity, researchers developed a sandwich ELISA method. MUC1 plays a dual role in tumor initiation and progression [10]. On one hand, MUC1 itself can mediate anti-tumor immune responses, making it a target molecule for tumor biomarker diagnosis and treatment. On the other hand, abnormal expression and disrupted polarity of MUC1 affect the intercellular adhesion between surface molecules, allowing tumor cells to evade immune surveillance.

The researchers employed the sandwich ELISA method with dual monoclonal antibodies to detect the levels of MUC1 protein in peripheral blood of patients with adenocarcinoma. Firstly, diluted rabbit polyclonal antibodies against MUC1 are added to each well and incubated overnight to immobilize the antibodies. Subsequently, 2% BSA is added to each well to block non-specific binding sites. Following this, the wells are incubated with different concentrations of MUC1 standard and serum samples from patients with adenocarcinoma and healthy controls (including negative controls). After a specified incubation period, the plate is washed, and detection antibodies (polyclonal antibodies against MUC1 in rats) and enzyme-conjugated secondary antibodies (sheep anti-rat HRP) are added. After another round of washing, the plate is developed using OPD substrate solution for color reaction. Finally, the reaction is terminated by adding 2M H₂SO₄, and absorbance values are measured at a wavelength of 490 nm using an ELISA reader. A standard curve can be used to analyze the concentration of MUC1 protein in the serum samples [9].

The experimenter concurrently utilized a commercial CA15-3 assay kit to measure the levels of MUC1 protein in the real serum, compared to the established sandwich ELISA method employing dual antibodies. The specific steps of the assay are as follows: Initially, the patient serum was diluted at a ratio of 1:50, and both the diluted serum samples and CA15-3 standard were added separately into the pre-prepared microplate. Upon completion of the incubation, the incubation mixture was removed, and the microplate was washed multiple times with wash solution to ensure thorough removal of any remaining droplets, followed by gentle tapping and inverting of the microplate to

ensure complete elimination of residual moisture. Next, the CA15-3 detection enzyme antibody was added, followed by another incubation for one hour. After repeating the washing steps, substrate TMB was added, and the reaction was allowed to proceed for a specific duration. Lastly, stop solution was added to halt the reaction, and the absorbance value at 450 nm wavelength was measured using the ELISA reader within 30 minutes. The CA15-3 concentration in the test sample was calculated based on the standard curve. If CA15-3 \geq 35 Unit/ml, the result was considered positive [9].

The findings from analyzing serum samples of 30 breast cancer patients and 78 healthy individuals using both the in-house developed double antibody sandwich ELISA method and the CA15-3 assay kit indicated that the double antibody sandwich ELISA method exhibited a detection rate and specificity of 100%. On the other hand, the CA15-3 assay kit demonstrated a lower detection rate of 3.33% but maintained a specificity of 100% (Table 1).

Table 1. The results of the MUC1 protein detection in the serum of breast cancer patients using the sandwich ELISA method [9].

Categorization	Dual antibody sandwich ELISA		CA15-3	
	Number of cases	Positivity rate (%)	Number of cases	Positivity rate (%)
Breast cancer patient	30	100(30/30)	30	3.3(1/30)
Benign Breast Diseases patient	18	22.2(4/18)	18	0.0(0/18)
healthy individual	78	0.00(0/78)	78	0.0(0/20)

6. ELISA-based adenosine deaminase detection in serum and saliva

DADA2 is a genetic immune deficiency disease caused by mutations in the ADA2 gene. The individuals afflicted by this ailment display manifestations of early-stage systemic inflammation, combined lymphocyte deficiency, and various health issues. ADA2 has potential associations with multiple diseases, such as HIV and AIDS, active tuberculosis, systemic lupus erythematosus and rheumatoid arthritis, liver fibrosis, breast cancer, and ovarian tumors, among others [11]. In essence, DADA2 is intricately linked to immune system regulation and the development of multiple diseases.

The concentration of active ADA2 was measured utilizing the ELISA technique by the researcher. They coated the ELISA plate with polyclonal antibodies that specifically bind to ADA2, incubating it overnight at 4°C. The plate was incubated at room temperature with shaking for one hour to allow for specific binding between ADA2 and the antibodies. By employing a standard curve generated through recombinant protein, the researchers were able to determine the concentration of target molecule in the samples [11]. The researchers also employed the ELISA technique to quantify the concentration of adenosine deaminase 2 (ADA2) activity. By utilizing a standard curve generated from wild-type recombinant protein, the researchers were able to determine the total protein concentration of ADA2 in the samples. Overall, this study reliably measured the concentration of ADA2 activity using the ELISA technique. Through this approach, the researchers obtained crucial data regarding the potential applications of ADA2 in disease diagnosis and treatment, and furnished a robust tool for further investigating the role of ADA2 in disease mechanisms [11].

Previous studies have indicated that the activity of ADA in the saliva of patients with various types of cancer is significantly lower than that of healthy control group [12]. Upon these discoveries, the researchers have decided to analyze the concentration of ADA2 in saliva samples of patients with head and neck cancer. Despite similar ADA2 concentrations between laryngeal squamous cell carcinoma patients and the control group, other groups of patients showed significantly higher ADA2 concentrations. Specifically, patients with laryngeal and tonsillar carcinomas exhibited the greatest disparity in ADA2 levels. The researchers conducted further analysis on saliva samples from patients with laryngeal, oropharyngeal, and tonsillar carcinomas. A significant increase in ADA2 concentrations was observed in all cancer patients, both those receiving treatment and those not yet

treated, indicating ADA2 as a promising predictive biomarker for these types of cancers under investigation [12]. Overall, the application of ELISA technique enabled the reliable measurement of the concentration of active ADA2 in saliva samples from head and neck cancer patients. This study provides a robust tool for understanding the potential role of ADA2 in cancer development and offers preliminary evidence of its potential as a cancer prognostic marker.

7. Conclusion

This research explored and synthesized the applications of ELISA for the detection of tumor biomarkers. This research introduces the fundamental principles of ELISA, provides an overview of tumor biomarkers, and discusses the application of ELISA in various research cases. ELISA technology plays a crucial role in the early diagnosis, treatment monitoring, and prognosis assessment of tumors as a widely utilized method for tumor marker detection. Through the use of ELISA, it is able to accurately and sensitively detect and quantify various tumor biomarkers, providing essential information and guidance for clinicians. The researchers successfully established a sandwich ELISA method utilizing dual antibodies for the detection of endosialin/TEM 1 (CD248) and MUC1 proteins, two tumor-associated markers. Through these studies, a significant upregulation of these markers in tumor patients was discovered, suggesting their potential as reliable tumor biomarkers for detection and screening purposes. Furthermore, the researchers also quantified the concentration of adenosine deaminase in blood and saliva using the ELISA method, offering a convenient and reliable approach for non-invasive biomarker detection in clinical settings.

In conclusion, ELISA holds a vital position in tumor biomarker research and clinical applications as a widely utilized technique. Although this article only highlights a fraction of ELISA's applications in tumor marker detection, its potential and versatility warrant further in-depth research and implementation. Future studies can continue to explore ELISA detection methods for other tumor markers and further optimize the sensitivity and specificity of the technology, advancing the field of oncology and providing improved diagnostic and treatment strategies for patients.

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