ELISA IN DETECTION OF CANCER

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Abstract

The diagnosis and detection of cancer at earlier stages is very important for the management of cancer. The presently available diagnostic blood tests are generally based on detecting the markers associated with tumors like for gastrointestinal cancer CA19-9 the cancer antigen, carcino-embryonic antigen (CEA), for breast cancer CA15-3 and for ovarian cancer CA125. However, these markers lacks specificity and sensitivity which prevents their use in the screening of cancer. Therefore new improved diagnostic techniques are required for the detection of cancer. The present review deals with modification of ELISA (Enzyme linked immunosorbant assay) in the detection of cancer.

Introduction

ELISA stands for enzyme linked immunosorbent assay which is based on the principle of antigen –antibody precipitation. It offers lot of advantages to the user over other radio-immunological techniques and especially on IRMA (immuno radiometric assay) as for this not much expense is required, reagents have much better shelf life, gives much more sensitive and precise results and above all used in both medical and veterinary sciences. There are different kinds of ELISA and all these ELISA are used for various applications. Here are shared some of the applications and modifications including parent technique for the detection of cancer (Paré and Hiet-ala, 1995)

Nano ELISA and Cancer

Conventional ELISA takes lot of time especially sandwich ELISA. So it is futile to use for the detection of cancer. In Nano ELISA one can use nanoparticles for detection of cancer receptors which express themselves in low quantities making them difficult to detect by conventional technique. Nanoparticles used such as of gold enhance the surface area of detection. Earlier usage includes making nanoparticle – antibody –HRP complex where labeled antibodies are made to fuse with the nanoparticle. This nanoparticle would be binding to the tumor receptor and the horse radish peroxidase will react to the chemicals around the area producing colored substrate. This modification although enhanced the sensitivity but not the cost which was used to create labeled antibody. One of the common proteins which are used as diagnostic tool for detection of cancer is p53 which is actually a protein which is tumor suppressor. When there is a development of cancer the concentration of p53 gets increase by the body (Soussi and Wiman, 2007). The increased concentration can be detected for deducing development of cancer. Earlier technique for p53 included using electrodes on which p53 specific DNA was immobilized in order so that p53 can bind after the application of charge (Marquette et al., 2006). The technique compromised with cost as well as sensitivity. Nano ELISA reduced the shortcomings. Procedure included initial activation of micro magnetic particles with the corporation of carbodiimide hydrochloride and allowed to incubate with the protein specific antibody. Latter include several washing steps and treatment by PBS.
including characterization of probes. Now gold nano particles were characterized by pH stabilization (Fayazfar et al., 2014).

Gold nanoparticles can also be prepared in laboratory by mixing it in distilled water and trisodium citrate solution. Slowly and slowly when it was mixed it formed molecule showing color transition from yellow to red confirming the formation of nano particle. Then nano particle was allowed to conjugate with antibody. The gold solution was centrifuged to get the pellet in which protein solution containing the antibody was mixed. The solution was mixed by keeping it on magnetic stirrer and for stabilization purpose poly ethylene glycol was added. After the successful immobilization, the complete (gold-antibody –HRP) complex was purified by treatment of PBS. For confirmation the complex was visualized by transmission electron microscopy. Later the efficiency was also checked by treating the complex with tetramethyl benzidine. The resultant chromogenic substrate was analyzed by spectrophotometer at 450 nm wavelength. The absorbance value confirmed the activity of HRP. Now to quantify the HRP which are bound to gold particles serial dilution technique was performed and later analyzed by spectrophotometer. After the preparation of reagents immunoassay was performed. Micro magnetic particles that were allowed to make complex with p53 antibody was allowed to mix with the p53 molecule i.e. antigen. This complex was then mixed with the nanoparticle probe. As a result probe and micro magnetic particle made a complex with each other which was kept in magnetic stirrer and then mixing with TMB and spectrophotometer reading at 450 nm brings out the last step of the protocol.

It is reported that classical technique takes 6 hours whereas modern technique takes 2 hours which shows that usage of nanoparticles in the technique significantly reduces the time by four hours. Without the usage of nanoparticles, antibody has less surface to get attach with but nanoparticles provide enough surface to make more than 1 antibody to attach to HRP , making technique much more sensitive. The optimum size of the nanoparticle is much more important for better efficacy. (10 nm particles were found to be effective than 15 and 30 nm in case of p53 protein detection). For this reaction 500 nm of gold nanoparticle probe and 1.5mg/ml of micro magnetic particle was optimum for efficient results. The ratio of concentrations of different components also determines the quality of test. It is found that 5 to 6 molecules of HRP can bind singly to gold nano particle and 3 molecules of antibody if both are not together used. For creation of complete complex 1-2 molecules of antibody and 5 molecules of HRP per 1 molecule of gold is sufficient. This technique can detect the protein to very minute quantities of 0.125ng/ml. The instrumentation required for this technique is same as conventional technique ensuring its wider applicability for serological testing (Jia et al., 2009).

**BEAD BASED ELISA FOR DETECTION OF OVARIAN CANCER**

Markers act as diagnostic tool for detection of diseases. For early diagnosis of cancer researchers are trying to find out various markers for various kinds of cancers. More identification of markers enables better detection as well as better strategy for treatment. For example two of the molecular markers CA125 and HE 4 found to act as molecular marker for ovarian cancer. Now the ideal marker is considered to be one, which can enable to detect cancer at very early stage even before the latter stage of first stage (Bandera et al., 2003). The minimum time required for detection of cancer by the aid of identification marker is termed as lead time. Less the lead time more efficient the marker is. Not even the time but the quantity of specimen required for detection is also very important. It is important for having a cost effective strategy for early detection of marker which responds at early stage of tumor. An exclusive quality of an efficient strategy is that it also enables to analyze the molecular communication of the marker with other markers.
Bead based ELISA method is perfect answer to that (Nifli et al., 2006). In bead based assay the antigen or molecular marker protein is sandwiched on the surface of beads instead of glass surface. Procedure is as follows –

The initial step includes selection of the primary antibody which will be binding to the surface of bead. The binding was confirmed and to check the reproducibility, the antibodies (two for CA125 and one for HE4) will be checked. As the bead based technique depends on studying the interaction of different markers correlation and coefficient of variance is calculated. After the preparation and selection of antibody, filter plates is used for conduction of complete assay. The assay can be performed at room temperature and without vacuum conditions. The stored antibodies are allowed for activation with vortexing and centrifuging it in decreasing order of step in a sequence. Addition of antibody to the bead started the main step where the complex stabilization was confirmed by treating the complex with certain complex of sulfur and carbon. After the successful completion of probe preparation the probe is allowed to make complex with (CA-125 and HE 4) proteins of interest and analyzed for results. Marker associated proteins were also analyzed in combine manner incorporating the same probe (multiplexing). Statistical methods including variance and others including correlation is conducted for comparison of the test sample with the standard solution.

Later the complex is analyzed by a fluorescent reader in order for determining mean fluorescence intensity. It is important to know the optimal conditions for the probe generated in order for efficient detection and testing. The validation of antibody and bead complex is done by the aid of biotinylation with the testing antibody generally isolated from animal serum (goat). There are various advantages of bead based detection of marker. On the top that it requires very less amount of sample. The procedure is highly reproducible. It is found that multiplexing of CA125 and HE 4 confirmed the cross reactivity of both the markers. Generally it is seen that in tumor development one or more markers and receptors are interlinked. Accuracy of the assay is without a question as experimentation suggests that the results between testing on tumor and non tumor samples providing clear cut differences. It offers consistent advantage over radioimmuno assay(Scholler et al., 2006)

**MICROARRAY ELISA FOR BREAST CANCER**

In case of breast cancer also detection can be done by the aid of microarray ELISA (Witzel et al., 2010). Protein microarray based ELISA is also used for determining quantity of proteins in unknown samples (Pin et al., 2016). In breast cancer hepatocyte growth factor gets increased in the body beyond the normal concentration. In the procedure microarray is prepared at the initial step. After the preparation of microarray incubation is allowed by addition of serum. Serum antibody will be attached which will allowed to make complex with biotinylated antibody. Later the complex is amplified by the aid of biotin and streptavidin is added as a substrate to get a colored product. In this cancer also multiplex experiment was performed between parent component HGF, vascular endothelial growth factor and PSA. The technique is so sensitive that it can detect HG F to concentrations of 0.5pg/ml (Woodbury et al., 2002)

**CONCLUSION**

ELISA is widely used for molecular diagnostics. In terms of cancer it is developing and modifying itself for bringing more accurate, precise and faster results. In the process new modifications are coming improving the parent technique.
REFERENCES


