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A Review of Enzyme Linked Immunoabsorbent Assay (ELISA) and Chemiluminescence Immunoassay (CLIA) Technologies

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ABSTRACT

Immunoassays are powerful analytical tools widely employed to detect and quantify analytes, primarily proteins, in biological fluids such as serum, urine, saliva, and sweat. Among the most common types are enzyme-linked immunosorbent assay (ELISA) and chemiluminescence- operational efficiency. ELISA, first introduced in the 1970s, has become a cornerstone in diagnostics and research due to its cost-effectiveness, ease of use, and adaptability to a wide range of targets. However, ELISA systems are often limited by their detection sensitivity for low-abundance analytes. To address this, more advanced immunoassay systems such as chemiluminescent immunoassay (CLIA) and electrochemiluminescence immunoassay (ECLIA) have emerged, offering enhanced detection capabilities. This paper outlines the evolution of immunoassay platforms, comparing traditional ELISA techniques with modern commercial systems including Fluorescent Polarization Immunoassay (FPIA), Enzyme Multiplied Immunoassay Technique (EMIT), Cloned Enzyme Donor Immunoassay (CEDIA), and Luminescent Oxygen Channeling Immunoassay (LOCI). Furthermore, it delves into the underlying mechanisms of CLIA, highlighting biologically derived chemiluminescent agents, such as firefly luciferase and aequorin, as well as non-biological agents like acridinium esters. Novel approaches including magnetic bead-based CLIA, flow injection systems, and capillary electrophoresis-based assays are also discussed for their contributions to improving automation, sensitivity, and speed. Special attention is given to the ADVIA Centaur XP system, a fully automated CLIA platform, for its clinical utility and scalable laboratory applications. This review consolidates the current landscape of immunoassay technologies and emphasizes their growing relevance in clinical diagnostics, pharmaceutical monitoring, and bioanalytical research.

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Introduction

An immunoassay is a test used to detect the presence or concentration of a molecule in a solution, and is carried out with the help of an antibody (in most cases) or an antigen (rarely). The molecule detected by the immunoassay is often referred to as an 'analyte'. Although, the analyte is usually a protein in nature, it may sometimes consist of different molecules, of varying sizes and types. Biological liquids such as serum, sweat, saliva or urine are frequently measured, for the purpose of detecting analytes, using immunoassay, for research purposes. Immunoassays occur in many different formats and variations.

Enzyme immunoassay (EIA) is popularly known and was introduced to represent immunoassays that would employ the use of enzyme-labeled antibody/antigen to detect antigen/antibody. These assays have multiple uses in the field of biological sciences and bio-analytics. One of the most common enzyme immunoassays that are primarily in use in this field is the enzyme-linked immunosorbent assay (ELISA) and was first introduced and developed by Engvall and Perlmann in 1971[1]. ELISA has wide applications that are not limited to the fields of human biology, immunology, pathology, plant pathology, and the food industry. Yet one of its greatest drawbacks is its inability to detect increasingly smaller amounts of target molecules. To overcome these limitations, newer more sensitive assays have been developed that make use of techniques such as fluorescence, chemiluminescence, and electrochemiluminescence. In 1985, Woodhead developed the chemiluminescence immunoassay (CLIA) and this assay directly labeled antibody using chemiluminescent indicators such as luminol, isoluminol, an acridinium ester. However, this assay is limited due to the technique that requires a relatively short duration of light output thus requiring the assistance of a robot. On the other hand, Electrochemilumiscent Immunoassay (ECLIA) combines the analytical advantages of chemiluminescent analysis in conjunction with the controlled operation by applying an electrode potential [1-6]. ECLIA replaces the conventional immunoassay systems and acts by converting electrical energy into light.

ELISA Systems

Enzyme Linked Immunosorbent Assay

Immunoassays (IAs) were first used in 1995, over the years, they have been developed and aid in various settings like clinical diagnosis, biopharmaceutical analysis, environmental monitoring, security, and

food testing [7,8]. They provide the qualitative, semiquantitative or quantitative detection in complex sample matrices which include bodily fluids like urine, blood, saliva, sweat, and vitreous humor of the eye [8-10].

The indispensable role that IAs play in the healthcare sector has led to advances in IA formats, of these formats, among the first to be used in the healthcare sector were radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) [11-13].

The ELISA format, because of its sensitivity, specificity, and precision, soon came to be the gold standard for diagnosis, pharmaceutical drugs and for toxic disease reports. As the years moved ahead, tremendous innovations were seen in the ELISA technology in terms of technology, features, and cost-effectiveness [14-17]. Currently, ELISA is deviating towards the wash-free ELISA Handbook of Immunoassay Technologies, critically reducing its duration and complexity.

Antibodies are a collection of glycoproteins that are a part of the Immunoglobulins group, that particularly bind to antigens [18-21]. Each antigen may have single or multiple binding sites on it, depending on its shape and size, where it binds with the antibody, this site is known as an Epitope. The binding of proteins that has occurred is reflected by the intensity of color change which is measured by light absorbance.

Immunoassay reactions usually occur in two types; they are competitive inhibition and/ or non-competitive inhibition, which is also known as the sandwich technique [22-25]. Competitive immunoassays require only one antibody for the reaction to occur and are commonly used for assays of small molecules such as therapeutic drugs or in cases of drug abuse. The sandwich technique requires two antibodies that are more commonly used for assays of relatively large molecules [26-28].

Homogeneous immunoassays generally do not require any separation and can find distinction between the various labels for detection, in comparison to Heterogeneous immunoassays that that generally require this. Homogeneous immunoassays eliminate the wash step and are compatible with most automated chemistry analyzers, thus making them easier to use. Heterogeneous immunoassays, however, possess the major advantage over homogenous immunoassays, that they are more sensitive than homogenous immunoassays.

Commercially Available ELISA Systems

The various commercially available ELISA systems are:

- **Fluorescent Polarization Immunoassay (FPIA)** produces a signal only if there is a final binding between the labeled antigen and the antibody molecule [29-32]. It is based on the principle of an assay that is highly competitive and works on the signal rendered by the fluorescence polarization. A miniscule molecule, in the system, when attached to the antigen molecule displays a Brownian motion that varies in comparison to when the label is conjugated to a larger antibody (140,000 or more Daltons). The intensity of the signal is reversely proportional to the concentration of the analyte.
- **Enzyme Multiplied Immunoassay Technique (EMIT)** is also a homogenous competitive immunoassay, that uses the enzyme glucose 6-phosphate dehydrogenase to label the antibody, this enzyme acts by reducing nicotinamide adenine dinucleotide (NAD) to NADH and the absorbance is monitored at 340 nm. Therefore, signal intensity in this system, however, is directly proportional to the analyte concentration [33-37].
- **The Cloned Enzyme Donor Immunoassay (CEDIA)** is a method that uses recombinant DNA technology in which bacterial enzyme beta-galactosidase is genetically engineered to form two inactive fragments. It acts by producing a signal when both the fragments combine. This signal is proportional to the analyte concentration [38-40].
- **Kinetic Interaction of Microparticle in Solution (KIMS)**: this system is based on the principle that in the absence of antigen molecules free antibodies bind to drug microparticle conjugates which form particle aggregates and lead to an increase in absorption, which is then optically measured at various visible wavelengths (500-650 nm) [41].
- **Luminescent Oxygen Channeling Immunoassays (LOCI):** this system is irradiated with light which generates single oxygen molecules in microbeads ("Sensibead") that are coupled to the analyte. When this binds to the respective antibody molecule, which is also coupled to another type of bead, it reacts by forming singlet oxygen and chemiluminescent signals are generated that is proportional to the concentration of the analyte-antibody complex [42-44].

CLIA Systems

A brief outline of the principle of Chemiluminescent Immunoassay is the emission of light that is produced when a chemical reaction occurs. This is particularly helpful in detection with little to no background interference and will also potentially serve as a more efficient alternative because of its low limits of detection, although, the reactions are difficult to perform as the have to be controlled very carefully. The applicability of this method, in the case of homogeneous immunoassays is not always possible since it is not modified by antibody binding. Heterogeneous CLIA has an increased sensitivity in comparison to homogenous mode and is hence is more commonly used [45,48]. It uses a chemiluminescent reagent or an enzyme for the marking of an antigen or antibody [45-49]. Once the reaction occurs, it leads to light emission, and the intensity of the light thus emitted is directly proportional to the strength of the analytes in the obtained sample.

Biologically Derived Chemiluminescence Agents

Chemiluminescent systems can be performed by means of chemically manufactured compounds as well as biological reagents. Examples biologically derived reagents may include:

- **Apoaequorin:** Which is a protein that is derived from the bioluminescent jellyfish species Aequorin, the underlying mechanism of which is believed to be activation by coelenterazine (acting as a substrate) and require the presence of calcium ions, leading to light emission occurring at 469 nanometers.
- **Firefly Luciferase:** Which is the enzyme responsible for the bioluminescence of fireflies and clicks beetles, activation of which occurs in the presence of ATP by oxidation, requires D-luciferin to act as a substrate and emits light at 560 nm.

The chemically manufactured (nonbiologic) reagents comprise of isoluminol and acridinium esters. Isoluminol causes emission of light at 425nm for increased durations of time by acting in the presence microperoxidase (catalyst) and undergoing oxidation. Acridinium esters are extremely sensitive agents and is usually used, they are active in the presence of hydrogen peroxide by undergoing oxidation in an alkaline solution and thus causing production of a brief flash of light which occurs at 429 nm. Numerous drugs like carbamazepine, phenytoin, phenobarbital, and valproic acid are tested using the CLIA systems. These techniques can also be combined with other detection systems like Electrochemiluminescence for increased sensitivity of this system. The substrates as well, may be combined

with various enzymatic labels for the enhanced advantage of amplification effects which leads to an inherent increase in sensitivity of chemiluminescence, an example of which is known as AMPPD (disodium 3-(4-methylspiro- [1,2-dioxetane-3,20 -tricyclo- [3.3.1.1] decan]-4-yl) phenyl phosphate). The AMPPD substrate is currently being employed for the testing of cardiac markers and endocrine markers. Dyes which are fluorescent in nature, such as Cy5, and Au nanoparticles (AuNPs), and quantum dots (QDs) have recently found their use as agents or labels in the CLIA systems of Immunoassay.

Non-Conventional Methods in the CLIA Systems

The non-conventional methods in the CLIA systems include three main categories:

- Magnetic Beads CLIA
- Flow Injection CLIA
- Capillary Electrophoresis for CLIA

Magnetic Beads (MBs) departures from the conventional approach of CLIA and has established many advantages that have proven to be useful for food safety, clinical diagnosis as well as environmental monitoring [50]. The principal method of analysis applied here comprises of the immobilization of antibodies that are present on the surface of the Magnetic Beads with a functional group. The advantages of this system include:

- It allocates the free suspension in the immunoreagents or rapid immunoreaction for the chemiluminescent substrate and thus helps in the occurrence of rapid reaction,
- The provision of a large surface area for binding more capture antibodies and thus it accelerates the reaction and also aids in sensitivity enhancement,
- it provides quick and easy isolation in the magnetic field, and
- the efficient concentration of even trace amounts of analyte is observed when the MBs are collected.

Flow Injection CLIA the combination of CLIA with flow injection analysis is quite convenient in practice and has ascertained the possibility for development towards automatic or automated CLIA techniques [51,52].

- In this method, the separation step is usually aided by a support-based immunoreaction. One of the key steps in this technique includes the selection of suitable solid support for the immunoreaction while developing a flow immunoassay. The reagents used for support most often in flow injection immunoassay for immobilization of antibodies or antigens are membrane materials or beads [53-55].
- The membrane materials used to perform flow injection CL immunoassay include cross-linked chitosan membrane, three-dimensional nanoporous silica dioxide film, aldehyde-activated polyethersulfone membrane, graphene films on nanosheets, and nanotubes made from carbon.
- The interpretation of flow injection CL immunoassay for protein marker detection has used silica, agarose, sepharose, polystyrene, resin, and glass beads.
- This technique conglomerates various advantages which include increased speed, precision, selectivity, and sensitivity.
- This technique allows the performance of both homogeneous and heterogeneous systems while being especially more appropriate for heterogeneous systems for the reason that the step of separation can be performed conveniently online in the flow injection technique[56].
- With the immense development of this technique in recent years, it is now possible to apply this technique for Detection of Protein Biomarkers, pharmaceutical analysis, and clinical diagnosis.
- Automated CLIA techniques in conjunction with flow injection analysis are combined for optimal performance which include small sample-cost, satisfactory reusability, worthy reproducibility, reduction in sample handling, and diminutive time.

Capillary Electrophoresis for CLIA has been thoroughly used as a tool for power separation as it has a significant scope for more efficiency in increased separation as well as increased specificity thus making it a powerful assay technique for the separation and analysis of biological samples [57-59].

- The advantages of this technique include high sensitivity, ease of operation, requirement of fewer samples, improved speed of assay, inexpensive apparatus, and ease of automation. Owing to these advantages capillary electrophoresis-based CL immunoassay has made it another increasingly popular method over the past decade.
- Its many uses comprise of successful application in the determination of tumor markers, hormones, and abuse drugs.

Automated Clia System

The **ADVIA Centaur XP** is a fully automated random-access immunoassay system, based on the use of chemiluminescent tracers and paramagnetic (MBs) particle solid-phase reagents. This system uses common reagents with the ADVIA Centaur and ADVIA Centaur CP immunoassay systems which were developed by Siemens Healthcare Diagnostics [60-63]. It provides a useful framework for examining how analyzers provide an extensive assay menu inclusive of infectious disease testing which is fully automated. It was first introduced in March 1998 and since that time have been expanded and improved with subsequent trials by obtaining adequate information. ADVIA Centaur CP was launched in 2005 and ADVIA Centaur XP was launched in 2006, this system has proven to be a better approach producing more consistent results with significant enhancement in productivity and efficiency. At present day, the ADVIA Centaur portfolio offers immunoassay testing solutions for small-sized laboratories as well as very large laboratories. This system has a user-friendly interface at the level of global markets. All system supplies can be changed during operation, without any need to pause or stop processing, which aids in high system productivity.

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