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# POTENTIAL APPLICATIONS OF THE CHEMILUMINESCENT METHODS IN TUMORAL DISEASES INVESTIGATION

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## ABSTRACT

Nowadays, in the clinic routine, protein biomarkers of efficacy, or mode of action, are used to better monitor and predict patient responses to drug treatment and diagnosis. Currently, the chemiluminescence biomarkers are applied for validation methods and screening. Here, was revised the development of the new analytical tool through immunological (antibody) and chemiluminescent methods combination, named chemiluminescence immunoassay (CLIA). In the tumoral process, increased expression of specific antigens is associated in patients with certain tumors. Thus, in our laboratories CLIA has been tested to study different tumoral lesions from glandular tissues, like prostate and thyroid. In conclusion, there is a great opportunity for introducing the adaptive chemiluminescence devices, since clinical diagnostics represents a huge, well-established and important analytical field.

## KEY WORDS

Chemiluminescence, tumoral diseases, antibody, acridinium ester

## INTRODUCTION:

In the last 15 years, the utility of luminescent labels has steadily increased. The phenomenon has been applied to biomedical science in immunoassays, DNA probe assays, and measurement of important enzymes and metabolites.

By developing chemiluminescent (CL) compounds that can be used as substrates for enzyme labels it is possible to increase the speed of ultrasensitive CL immunoassays without impairing the sensitivity.

The term “chemiluminescence” was first coined by Eilhardt Weideman in 1888. This reaction refers to the emission of light from a chemical transformation. Chemiluminescent reactions emit light of varying degrees of intensity and lifetime, with colors that span the visible spectrum[1,2].

In 1998 Robert Wilson of Liverpool University Chemistry Department was looking at similarities between simple iron containing compounds and enzymes such as horseradish peroxidase (HRP). During this work he acquired a group of compounds called acridan esters from the US company Lumigen. Works had shown that acridan esters could be used as a chemiluminescence substrate for HRP, and Wilson demonstrated that light-emitting reactions could also be triggered electrochemically. Acridan esters are oxidized to more familiar acridinium esters that have been used as labels in ultrasensitive chemiluminescence immunoassays and nucleotide assays for many years[3,4].

Chemiluminescent reactions occur in the gas, solid and liquid state. In its simplest it can be represented as:

$$A + B \longrightarrow [I] \longrightarrow \text{products} + \text{light}$$

where [I] is a highly energetic intermediate compound produced from a chemical activation reaction when two reagents (A and B) are mixed. The [I] is short-lived and returns to a lower energy state by emitting visible light[1,5].

Quantitative analysis of chemiluminescence images, performed by defining suitable areas and evaluating the total number of photons that fluxes from whitin those areas, permits the quantitative assessment of distribution of light emission. The luminescence intensity is usually expressed as photons per second and surface area or in arbitrary units (often called relative light units, RLU), depending on the instrument used. Nevertheless, absolute quantitative analysis is hampered by several factors, including the lack of suitable calibration systems, the need for standardized and reproducible experimental conditions, and the influence of the sample properties on the emission intensity[6].

There are many systems of chemiluminescence of which the two most widely used are the luminal based and the peroxy-oxalate based systems[7].

In general, the luminol or acridinium ester is a major chemiluminescent analytical technique is used in clinical analysis[8]. As effective CL labels for biomolecules, acridinium esters have received great interest for immumoassay and DNA assay[9].

In the ours laboratories we labeling acridinium ester molecule with Concanavalin A (ConA), to be used as auxiliary histochemistry tool to help the clinical-pathological evaluation of infiltrating duct carcinoma, a human mammary tumour of high incidence in the State of Pernambuco-Brazil[10].

The attractiveness of chemiluminescence as an analytical tool is the simplicity of detection. The fact that a chemiluminescent process is, by definition, its own light source means that assay methods and the instruments used to perform them need only provide a way to detect light and record the result. Luminometers need consist of only a light-tight sample housing and some type of photodetector. Taken to the extremes of simplicity, photographic or x-ray film or even visual detection can be used[11,12].

Chemiluminescence (CL) has been known to be a powerful analytical technique that exhibits high sensitivity and selectivity, and the simple requirements of chemiluminescent methods make them robust and easy to use[9]. The recent availability of high sensitivity and high-resolution light imaging devices at reasonable cost has led to an increased diffusion of CL imaging analytical techniques. This techniques take advantage of detection in comparison with other spectroscopic methods (high sensitivity and specificity, low background signal, easy quantitative analysis and wide dynamic range) and the possibility to localize and quantify the light emission on a sample down to the single-photon level[13,14].

The efficiency of a chemiluminescent reaction is given by the quantum yield, which is a measure of the fraction of reacting molecules that actually produce light. For analytical chemists the main attraction of CL is the opportunity to carry out sensitive assays over a wide range of concentrations using relatively inexpensive equipment. In practice it is usually combined with a complementary technique that confers specificity on the CL reaction. The most widely used complementary technique is CL immunoassay where antibody labels such as acridinium esters have been used to detect analytes at picomolar concentrations[3].

## LUMINESCENCE METHODS:

### *Chemiluminescence immunoassay (CLIA) methods:*

Immunoassays based on chemiluminescence have substantially greater sensitivity and dynamic range than those based on earlier-generation detection techniques. Efficient light emission with low background is coupled with the high sensitivity and broad range of the photomultiplier detector. For every photon of light striking the surface of the photomultiplier, there is a $10^6$ fold electronic amplification of the signal. Photomultipliers have very low background noise and inherent dynamic ranges of 5 to 6 orders of magnitude[15].

Chemiluminescence enzyme immunoassay (CLEIA), which integrates the advantages of immunoassay and chemiluminescence determination such as high specificity and throughput, rapidity and convenience in operation and relatively simple and inexpensive instrumentation[16,17].

The chemiluminescence imaging have also been extensively applied for the evaluation of the spatial distribution of a given target molecule, chemical or biochemical process on macro or microsamples associated with traditional methods, immunohistochemistry (IMH), in situ hybridization (ISH), enzyme or chemical reactions are used for the localization of antigens, gene sequences, enzymes or metabolites in cells and tissue sections[2].

Even though a wide range of different bio- and chemiluminescent systems have been applied in conventional chemiluminescence assays, only a small number of luminescent systems proved suitable for imaging applications. The main requirement, which is particularly crucial in imaging microscopy, is the localization of the luminescent signal in close proximity to the site where the luminescent reaction takes place[6].

For example, bio- and chemiluminescence imaging microscopy a target molecule is often detected through its binding to a biospecific probe labeled with an enzyme that catalyzes a chemiluminescent reaction. Accurate localization of the target, down to the micrometer scale, requires that the light emission take place close to enzyme label[17].

IMH is based on the use of highly specific antibodies, able to bind to an endogenous and/or tumoral antigen (usually

a protein), which are subsequently detected by means of class-specific antibodies conjugated to enzymes (Figure 1).

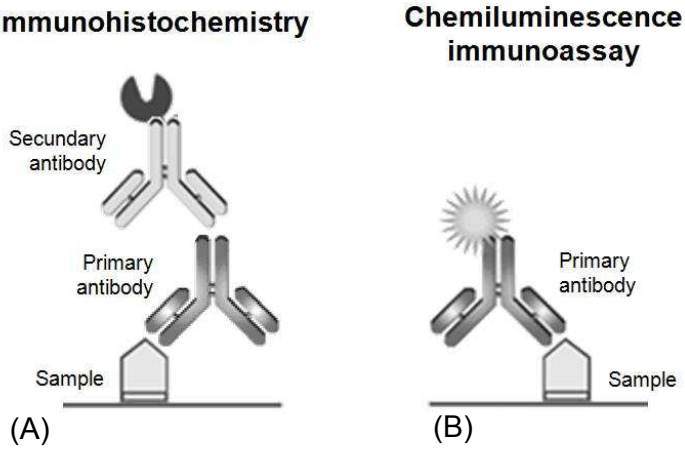


**Figure 1**
***Two immunoassay techniques. (A) Illustration of classical Immunohistochemistry (based on enzymatic reactions) and (B) Chemiluminescence immunoassay method.***

Now, the chemiluminescence immunoassay (CLIA) thus combine the specificity and sensitivity of labeled probes evidenced by chemiluminescence and spatial morphological resolution and localization of the signal of the IMH technique[18].

Among assay methods, chemiluminescence detection represents a versatile, ultrasensitive tool with a wide range of applications in biotechnology. It also gives a sensitive, rapid alternative to radioactivity as a detection principle in IA for the determination of molecules (e.g., proteins, hormones, drugs, nucleic acids and environmental pollutants). Chemiluminescence is now commonly used for immunoassay in the form of a CL label or as a CL detection reaction for an enzyme or a nanoparticle label. In recent years, CLIA has become very popular in clinical chemistry and environmental analysis, due to its high sensitivity, wide dynamic range and complete automation[19,20].

With the development and application of recombinant Ab (rAb) technology, markers and related techniques, solid-phase materials and improvements in automation, integration and miniaturization, CLIA has acquired an entirely new appearance[17].

Reagents required for reactions that produce CL may becoupled to Abs or antigens (Ags) and used as labels for immunoassay. Since the first report on CL labels in 1976, considerable efforts have been devoted to developing practical CL-labeling systems because of their low limits of detection (LODs) (Seitz, 1984). Luminol, isoluminol and its derivatives, acridinium ester, horseradish peroxidase (HRP) and alkaline phosphatase (ALP) have frequently been employed as CL labels in immunoassay for development and application of CLIA methods. Because the CL-detection methods have very low LODs, new CL labels and related substrates, new label technologies have been studied and obtained staggering results[12,22].

Scorilas and co-workers (2005)[23] synthesized two novel biotinylated acridinium derivatives, 9-(2-biotinyl-oxyethyl)-carboxylate-10-methyl-acridinium triflate (BOCMAT) and 9-(2-biotinyl-amidoethyl)-carboxylate-10-methyl-acridinium triflate (BACMAT), and described their luminescent properties and immunoassay applications.

***Biochemiluminescence (BCL):***

Owing to the light signal being generated by a chemical reaction in the dark, CL shows lower nonspecific signal and noninterference by light scattering. CL presents excellent performance in the mode of sandwich-type assays, the sensitivity of which is determined primarily according to the detection limit of the label. In the most format of CL immunoassay, the bound sample constituents are usually separated by an immobilized immunoreagent on a solid phase, for example, microwell plates, assay tubes and microparticles[24].

In the same way, the bound and free tracers can also be separatedand the separation step guarantees the low background signal of CL reaction.

## LUMINESCENT TUMOR MARKERS:

Despite years of research and hundreds of reports on tumor markers in oncology, the number of markers that have emerged as clinically useful is pitifully small. Often, initially reported studies of a marker show great promise, but subsequent studies on the same or related markers yield inconsistent conclusions or stand in direct contradiction to the promising results[25].

The proteins, mainly antibodies, are extensively used as diagnostic tools in a wide array of different analyses. Antibody-based immunoassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for investigation of biomolecules[26].

Light-emitting chemical reactions (chemiluminescence - CL) and biological reactions (bioluminescence BL) have a diverse range of analytical applications but relatively few have been adopted by routine clinical laboratories[2].

For example, the principle of CL has been employed in the field of Obstetrics and Gynecology for the early detection of cervical cancer and pre-cancer[6].

Tumor growth and metastases, as well as drug efficacy, have been monitored in living animals by injecting a mouse with luminescence recombinant tumor cells and imaging the produced light[27]. Alternatively, primary tumors and unknown metastases can be revealed in vivo by using engineered light-emitting cells as probes for tumor location[28].

The concentration of carcinoembryonic antigen (CEA) in serum obtained from different carcinosis patients was detected by using the method of double antibody sandwich CL immunoassay and the results obtained by this method are fairly well agreeable to those obtained by a consagrated detection method (RIA)[29].

Zhuang and co-workers (2004)[29] synthesized a new biacridine compound, 10,10-dimethyl-3, 3-disulfo-9, 9-biacridine (DMDSBA) as a CL label and established a sandwich CLIA method for the determination of carcino-embryonic Ag (CEA) in human serum for detection of tumoral diseases.

A chemiluminescence enzyme immunoassay (CLEIA) based on alkaline phosphatase ALP-labeling has been proposed for AFP detection. But ALP-labeling methodology shows two weak points when compared with HRP-labeling methods, which will bring high background and unavoidably, unproportionate or false positive results in the clinical usage[30].

Zhang and co-workers (2009)[31] pretreating the magnetic particles with fluorescein isothiocyanate (FITC) labeled anti-AFP monoclonal antibody (FITC-McAb), a one step CLEIA based on magnetic particles was developed for AFP with high simplicity and sensitivity, as well as wide linear range. The proposed magnetic particle based CLEIA was used to evaluate AFP in human sera samples and a good correlation was obtainedwhen comparing the results with that from a commercial electrochemiluminescence immunoassay kit.

The α-fetoprotein (AFP) is the most widely used tumor marker through CL for the diagnosis of Hepatocellular carcinoma (HCC)[32,33].

Despite the prostate cancer (PCa) has become a most widespread and stubborn disease and a major cause of death in the old age male population nowadays[34]. Zheng and co-workers (2008)[24] development sensitive chemiluminescence immunosensor was

developed for the detection of PSA. A sandwich assay format was established by using a monoclonal antibody pair acting as the capture probe and detecting probe, respectively.

Most of the current PSA detection methods are usually based on immunoassays. The more established approaches include enzyme-linked immunosorbent assays (ELISA)[35], time-resolved immunofluorometric assay[36], surface plasmon fluorescence immunoassay[37], bioluminescent immunoassay[38], electrochemical[39] and surface-enhanced Raman scattering (SERS)[40].

Lately, several new PSA detection methods employing the nanowire electrodes[41], the nanoparticle-based bio bar code[42], and the microcantilever method[43] are proposed. Although they all have their individual strengths, chemiluminescence (CL) is among the most widely used readout modality in virtue of undoubted advantages over other more widely used systems[12,44].

## PERSPECTIVES AND FUTURE:

Chemiluminescence is a well-established detection principle in various fields, including liquid chromatography, pharmaceutical and analysis, immuno- and gene probe assays. Nowadays, the CL represents a potent and versatile medical analytical tool suitable for a wide range of applications, because it combines the high delectability of the of the luminescence signal with the possibility to localize and quantify the light emission in a sample[6].

The high detectability and rapidity of CL techniques, along with the availability of microarray-based analytical devices, allows the development of high throughput screening assays, in which simultaneous, multi-analyte detection is performed on multi samples[17].

Therefore, more and more medical experts and chemists are interested in CLIA. However, the development of CLIA is dependent on the application of the sensitive and selective chemiluminescent probe[29].

Improvements in analytical sensitivity will likely lead to the discovery of new analytes tumours detection. Technical enhancement holds the promise of detecting very low concentrations in serum using nanoparticles as labels and CL detection[45,46].

In conclusion, chemiluminescent immunoassay (CLIA) is a fast and simple method without radioactive pollution, its sensitivity is usually higher than that of fluorescent immunoassay and enzyme immunoassay[29].

Nowadays, the method using nanoparticles, especially metal, as biological labels has attracted considerable interest. As biological labels, NPs present many advantages[45,46,47].

Recently in our laboratory we tested the applications of chemiluminescence immunoassay with galectin-3 acridinum ester conjugated to anti-Galectin3 antibody in prostatic and thyroid tumors and chemiluminescent assays with acridinum ester conjugated with Concanavalin-A in breast lesions.

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