Recent Applications of Chemiluminescence Assays in Clinical Immunology

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Abstract: Chemiluminescence has traditionally been used to study the nature of the oxidative bactericidal mechanisms of neutrophils and monocytes, intrinsic defects of abnormally functioning neutrophils or monocytes and cell activation. During the last ten years, Chemiluminescence has been applied in a wide variety of techniques, including immunoassays, protein blotting and toxicological and pharmacological tests (e.g. after exposure to antibiotic or immunomodulators agents, such as adjuvants and cytotoxic drugs). In this review, we discuss some promising clinical applications of Chemiluminescence in clinical immunology for the study of autoimmune diseases, inflammatory responses, endocrine disorders, immunodeficient states, mucosal immune responses against drugs and pathogens and host responses against tumors and infections. Further, we review the numerous advantages of Chemiluminescence-based methods over other methods to assay the same endpoints, which facilitate their use in the current practice of clinical immunology.

Keywords: Chemiluminescence assays, phagocytic activity, clinical immunology, tumors, immunodeficiency's states, inflammatory response, infectious diseases, endocrine disorders, cell activation.

INTRODUCTION

Chemiluminescence (CL) is defined as the emission of electromagnetic radiation caused by a chemical reaction to produce light (Table **1**) [1]. CL has traditionally been used to study the nature of the oxidative bactericidal mechanisms of neutrophils (PMN) and monocytes (MN), but a considerable number of patients who suffer from immunological disorders have benefited from recent applications of CL assays. Table **2** shows the advantages of CL methods over other conventional diagnostic methods developed during the 1990s [2-9]. CL immunoassays appear to be more sensitive than ELISAs and radioimmunoassays to determine a wide spectrum of analytes, and CL can be combined with molecular biology techniques to get an ultrasensitive assay. This review puts emphasis on the recent uses of CL methods to study immunological diseases and the advantages of their use.

CL ASSAYS TO EVALUATE CELLULAR ACTIVATION

CL has been used to study the nature of the oxidative bactericidal mechanisms of PMN and MN, the intrinsic defects of abnormally functioning PMN or MN, and the effects of various agents, such as drug and opsonins, on phagocytic cells [10]. Stimulation of phagocytes by several substances causes release of highly reactive oxygen species (ROS), [11] and CL can be inhibited by the superoxide scavenger, superoxide dismutase, and by selective inhibitors of the phagocyte NADPH oxidase. Further, a wide number of substances, such as cytokines, can modulate antigen-induced CL [12]. The production of ROS by MN and PMN has been widely assayed by the generation of CL in the presence of luminol (Fig. **1**). Luminol is a very lipid-soluble substance that can penetrate cells and tissue easily, and it is used in various ways to measure luminescence in single phagocytic cells, groups of cells, and cells bound to or located within tissue. The substances proposed to produce CL are singlet oxygen, superoxide anion, hydrogen peroxide, hypochlorous acid, and hydroxyl free radicals [13-15].

The initiation and resolution of inflammation is an area where CL techniques can be widely utilized. Towbin *et al*. developed a CL assay to examine how the outcome of an inflammatory reaction is intimately associated to the balance between IL-1 and IL-1 receptor antagonist (IL-1ra) cytokines. Further, the assay assessed the effects of anti-inflammatory agents on the expression of IL-1 and IL-1ra in bodily fluids [16].

The use of luminol-dependent CL may prove valuable as a method to measure the earliest events in the inflammatory process and may facilitate studying the mechanisms that produce inflammation. Luminol-dependent CL predominantly reflects the production of H_2O_2 together with Nitric Oxide (NO)/peroxynitrite formation [17-19].

A new assay has been recently used for the detection of endotoxin activity of lipopolysaccharide in whole blood based on *in vitro* neutrophil activation. This novel type of assay uses the priming effects of complement-opsonized immune complexes on the respiratory burst activity of neutrophils. The chemiluminescent emission was related to disturbances in the membrane NADPH oxidase and with myeloperoxidase activity of neutrophils [20]. Additionally, the NADPH oxidase activity, which is important to investigate the role of PLA2 products, could also be analyzed by CL assays in the presence of inhibitors of the arachidonic acid cascade [21]. and finally to study the kinetics and magnitude of the oxidative burst capacity of granulocytes from

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Table 1. Requirements for Chemiluminescence Emission

1. The reaction must be exothermic to produce sufficient energy to from the electronically excited state, can be established in terms of ΔG.
$-\Delta G \frac{hc}{\lambda_{ex}} = \frac{2.86 \times 10^4}{\lambda_{ex}}$ λex= long-wavelength limit for excitation of the chemiluminescent species.
2. The reaction pathway must be favourable to channel the energy for the formation of an electronically excited state.
3. Photon emission must be a favourable deactivation process of the excited product in relation to other competitive non-radioactive processes that may appear in low proportion.

This box was abstracted from the textbook. Chemiluminescence. Principles and Applications in Biology and Medicine. Chichester.
adapted from Campbell, A.K. *Chemiluminescence: Principles and Applications in Biology and Medicine*. Ellis Horwood series in biomedicine. New York. **1988**, [1].

Table 2. Advantages of Chemiluminescence-Based Assays in Conjunction with Molecular Biology Techniques in the Decade of 90´s

Author	Chemiluminescence-based assays
Bronstein, I., *et al*. (1994) [2]	Chemiluminescent assays have been described for the secreted alkaline phosphatase and beta-glucuronidase reporter gene products. These assays provide simple, sensitive, non-isotopic alternatives to existing detection methods and are performed in microplate or tube luminometers or in a scintillation counter
Barlet, V., *et al*. (1994) [3]	The chemiluminescent assay is easy to perform as a routine diagnostic procedure and may be a useful alternative to the radioactive solution hybridization method
Alkan, S., *et al*. (1994) [4]	The chemiluminescence immunoassays appear to be more sensitive than existing ELISAs or radioimmunoassays and may find new application areas
Martin, C. S., *et al*. (1995) [5]	Recently have been developed an assay system to accurately quantitate PCR products that utilizes solid-phase capture and an enzyme-linked chemiluminescent detection method. Biotinylated PCR products are quantitated by capture onto a streptavidin-coated surface, followed by hybridization of an internal fluorescein-labeled oligonucleotide probe and subsequent detection with an anti-fluorescein-alkaline phosphatase conjugate and a chemiluminescent substrate. The PCR-Light system was an ultrasensitive and rapid assay for PCR product detection
Bronstein, I., *et al*. and Martin, C. S., *et al*. (1996) [6, 7]	Chemiluminescence-based assays of reporter gene expression, have many biomedical applications, this detection technology permits the combined luminescence detection of two different reporter enzymes in the same tube, e.g., a dual assay for beta-galactosidase and luciferase. It increase sensitivity over those obtained with conventional colorimetric or fluorometric assays
Yang, T. T. *et al*. (1997) [8]	The cDNA encoding secreted alkaline phosphatase is a useful tool for investigating the function of known or putative enhancer/promoter elements. The chemiluminescence-based secreted alkaline phosphatase assay is about 10-fold more sensitive than similar assays using firefly luciferase as the reporter enzyme
Chen, T., *et al*. (1998) [9]	A DNA optical sensor system was proposed based on the combination of sandwich solution hybridization, magnetic bead capture, flow injection and chemiluminescence for rapid detection of DNA hybridization (e.g., bacterial alkaline phosphatase gene and Hepatitis B virus DNA could be used as target DNA)

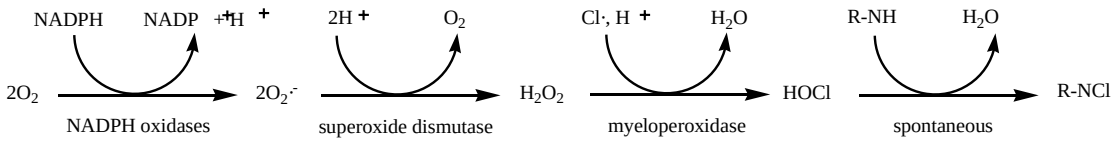

Fig. (1). The production of reactive oxygen species like anion superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) by neutrophils have been assayed widely by the generation of chemiluminescence in the presence of luminol.

peripheral blood, as early criteria of cell activation in order to evaluate their future uses in both: as immune modulator and future carrier for vaccinal epitopes [22, 23].

Whole blood CL allows for the evaluation of both the oxygen radicals released by phagocytes and the serum opsonin activity using very small amounts of blood. Some authors suggest that whole blood CL could be applied widely for the early detection of postoperative infections and to assess host defenses against infection [24].

CL ASSAYS TO EXPLORE AUTOIMMUNE DISEASES

The contributions of antibodies and immune complexes to neutrophil activation have been well established. It has important implications for monitoring patients who suffer

from autoimmune diseases [25]. Immune complex-mediated toxicity is known to cause tissue damage in patients who suffer from autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and vasculitis. Immune complexes have been found on the surface of polymorphonuclear leukocytes. CL can be quantitatively inhibited by the interaction of PMN with immune complexes or human gamma globulin [26]. On the other hand, fluid-phase IgG can act as a modulatory factor by inhibiting the generation of ROS by PMN, which are stimulated with precipitated IgG immune complexes, in a dose-dependent manner. It is associated to the ROS production induced by immune complexes during phagocytosis and the subsequent deposit of them on certain tissues [27].

Familial studies of autoimmunity have benefited by using CL assays. For example, CL assays were used to study the monocyte phagocytic system in children with rheumatic heart disease compared to their parents, their normal siblings and families without rheumatism. The study established a relationship between genetic patterns and phagocytic explosions to determine whether the activities of gene products that function in the monocyte phagocytic system are possible genetic markers for rheumatic susceptibility [28].

NO metabolism alterations have been described in neuroimmunologic disorders, and they impact the prognosis and subsequent treatment of the disease. In 1999, Hermanova evaluated chemical compounds, known to alter NO metabolism, for their effect on the adhesive properties of leukocytes and erythrocytes in the blood CL of multiple sclerosis patients compared with controls, and they found that the activity of the disease was related to NO-dependent alterations of the oxidative burst [29].

Regulation of ROS emissions has been implicated in tissue damage and in autoimmune disease because redox signaling pathways are implicated in cell growth, proliferation, apoptosis, cellular adhesion, extracellular matrix remodeling, phagocytosis and cytoskeletal reorganization. CL assays and PCR/RT-PCR techniques were utilized to determine the expression of the different components of the NADPH oxidase system in lens tissue. This study demonstrated that redox signaling might play an important role in how growth factors effect lens growth and development [30].

The activation of neutrophils may play a role in the increased propensity for bacterial infections in patients with primary Sjogren's syndrome. CL assays have been used to evaluate the predisposition to infections in such patients [31] and to study the relationship between metabolic ketosis and neutrophil microbicidal activity [32]. These CL assays can be used to decide whether to add immunomodulators to the patient or to reevaluate the control of the disease because changes, such as autoantibody production in Sjogren´s syndrome and ketosis in diabetic patients, can modify the CL response in neutrophils.

APPLICATION OF THE CL ASSAY TO STUDY IMMUNODEFICIENCY DISORDERS

The CL method helps to investigate the effects of highly active antiretroviral treatment on neutrophil and monocyte function in patients with moderately advanced HIV-1 infection. The functional activity of granulocytes has been measured by assessing chemotaxis towards a bacterial peptide and oxidative burst, as measured by CL production. CL was used to measure the chemotactic and fungicidal activity of patients compared with healthy controls. In this study, CL assays were used to evaluate the efficacy of a specific therapy, and similar approaches could be used to explore patients who do not respond to antimicrobial therapies [33].

The genotoxic effects in infants born from HIV positive women receiving antiretroviral nucleoside analogue drugs can be evaluated using a PCR- based CL detection method to determine the copy number of the mitochondrial D Loop gene [34].

While numerous primary immunodeficiencies remain unexplained in their etiopathogeny, CL assays have been used to study new alterations of the cellular response [35], which might contribute to pathogenesis of these diseases and help establish better treatments.

The reliability of methods for the measurement of chemotaxis is a common clinical problem. However, it has recently been shown that the number of cells migrating through a filter can be measured by determining their ATP content by CL [36]. This could be used to reduce the conventional rates to explore abnormalities in the chemotactic responses.

EVALUATION OF DRUGS AND CHEMICAL PRODUCTS THROUGH CL

The luminol-enhanced CL assay has been used to examine the effects of different concentration of antifungal agents (bifonazole, fluconazole, itraconazole, and terbinafine) on the CL response of mouse spleen cells. The reduction of the CL response is related to the inhibition of the cellular immune response [37]. Further, the effect of antifungal drugs on the phagocytic activity of PMNs, which protects against septic shock by inhibiting the PMN-mediated inflammatory cascade without compromising their phagocytic activity, can be evaluated by a CL assay [38].

Flavonoids, which are the main constituents of herbal medicines, have been reported to inhibit the growth of *Helicobacter pylori*. The technique of CL helps to evaluate the anti-HP activity of some flavonoids, such as flavanols, flavones, flavonols and isoflavonoids [39].

Neuramidinase inhibitors were recently licensed for the prophylaxis and treatment of influenza virus infection in humans. Recent data have shown that a CL assay is a rapid and reliable method for screening large numbers of influenza isolates for susceptibility to inhibitors [40].

Toxicity of chemical products on the vertebrate immune system is usually evaluated with CL. Recently, the effects of Permethrin, a member of the newest class of insecticides, was evaluated on splenic macrophages using this technique [41].

Antibiotics that can potentiate phagocyte functions may be more effective in the treatment of certain microbial infections than others. The measurement of superoxide anion

generation by neutrophils through CL assays helps to quantify this phenomenon [42].

CL assays can be applied to explore the immunogenicity and immune responses against adjuvants. For example, Masihi *et al*. used luminal-dependent CL to evaluate respiratory burst in splenic cells from mice pretreated with oil-in-water emulsions of muramyl dipeptide, trehalose dimycolate, or the combination of muramyl dipeptide with trehalose dimycolate in response to stimulation by zymosan. The authors concluded that the enhancement of nonspecific resistance to influenza virus infection was related to the chemical structure of the synthetic immunostimulant [43]. In similar way, Levamisole, an antihelminthic agent reported to nonspecifically enhance various parameters of the immune response, was examined for its effect on chemotaxis of human neutrophils and on the levels of cellular cyclic nucleotides [44].

APPLICATION OF CL ASSAYS TO STUDY INFECTIOUS DISEASES

Changes in immune function occur in elderly people, which increase their risk for infections and tumors. Braga *et al*. demonstrated a reduction in luminol-amplified CL in elderly subjects compared with younger controls. They studied whether the level of susceptibility to infectious diseases could be used to determine the use of vaccines and immunomodulators in different physiological conditions, such as being elderly [45].

Cellular immunity is primarily responsible for *Treponema pallidum* clearance and resolution. Aldinil *et al*. analyzed the interaction of *T. pallidum* with isolated rat Kupffer cells *in vitro* using CL to measure the production of ROS to assess the phagocytic activity [46].

A wide number of microorganisms cause important alterations in the phagocytic host response, and this is in close relation with latent infections and persistent states of unresponsiveness to drugs. Therefore, Carlyon *et al*. evaluated the effects of *A. phagocytophilum* infection on neutrophils' NADPH oxidase assembly and ROS production using CL. CL has been also used to demonstrate the impact of microorganism derivates on cell activation and the kinetics of apoptosis [47, 48], which are implicated in sepsis, multiorgan failure and immune recognition of foreign epitopes. The contribution of ROS to atherosclerotic development and the progression during *Chlamydia pneumoniae* infection was evaluated [49] using a CL test.

Opsonization is required to increase the phagocytic index and decrease the response time to an array of pathogens. The oxidative burst of neutrophils challenged with nonopsonized and opsonized microorganisms can be measured with luminol-enhanced CL. This technique is suitable for measuring the oxidative bursts of the nonopsonic phagocytosis by neutrophils [50-52]. Additionally, a luminol-enhanced CL assay can be used to investigate opsonic requirements for phagocytosis of bacteria serotypes [53].

The attraction of component cells to the zones of inflammation is a mechanism to control the inflammatory response. Many neutrophils are recruited into the intestinal lumen in response to pathogenic infections. Hofman *et al*. evaluated the effect of transepithelial migration on phagocytosis by evaluating immunofluorescence and superoxide production by PMNs using luminol-mediated CL [54].

The Digene Hybrid Capture System is a solution hybridization antibody capture assay used for the chemiluminescent detection and quantitation of cytomegalovirus DNA in leukocytes. The Digene Hybrid Capture System assay provides a rapid, quantitative, and objective measure of cytomegalovirus activity in leukocytes, even when the results do not always correlate with clinical disease [55].

Barbour *et al*. evaluated PMNs of 18 patients during episodes of active bacterial infection. They found that CL production remained increased with persistent infection but fell to the levels of controls with appropriate therapy. These findings were analyzed, in conjunction with the chemotactic response, to assess the activation state of neutrophils from patients suffering from active bacterial infection [56]. Solber *et al*. also demonstrated that the outcome of bacterial infections is closely related with PMN function because the majority of patients with bacterial infections have PMNs with normal or increased function, and a fatal outcome of the disease was more frequent in patients whose have reduced PMN function [57]. In the same way, using phorbol myristate acetate as a stimulus, leukocytes from diabetic patients had a markedly reduced CL response and reduced superoxide anion response, which may contribute to impaired bacterial killing and may explain, in part, their susceptibility to infection [58]. On the other hand, both C-reactive protein and ferritin have been reported to reflect the extent of oxidative stress and inflammation in individual patients and may be useful markers of disease activity and mortality risk. The intensity of lucigenin-enhanced CL is related with superoxide levels [59] and disease activity. These assays are recommended to evaluate the sepsis progression in susceptible patients.

Using patients with postoperative infections, M. Salo *et al*. evaluated the oxygen-dependent microbicidal responses of separated granulocytes to microbes isolated from each patient. They found that the decreased CL responses were probably due to the infection and reflect the depressed microbicidal capacity of granulocytes [60]. Khan, HA used a rat model of sepsis-induced alterations in both blood and peritoneal fluid PMNs. Their quantitative assessment may be helpful in disease evaluation and designing effective therapies [61] In addition, the activation of lipid peroxidation during episodes of infection were estimated by an increase in the rate of CL in peripheral lymphocytes [62].

Defect in oxidative routes of neutrophil metabolism and in Fc-receptors involved in phagocytosis are major determinants of susceptibility to infection, and such defects seem to be especially common in patients with severe 'multifocal infections' [63]. The luminal-enhanced CL of heterologous neutrophils was used to assess the capacity of *Haemophilus influenzae* type b-specific antibodies to induce opsonization of *Haemophilus influenzae* type b with autologous heat-inactivated sera from children immunized with the *Haemophilus influenzae* type b capsular polysaccharide-polyribosyiribitol phosphate-conjugate vaccine [64].

The clinical evaluation of a novel, fully automated CL immunoassay for the determination of immunoglobulin G avidity to human cytomegalovirus showed 92.8% sensitivity and 84.7% specificity in detecting a recent (≤90 days) primary human cytomegalovirus infection. The assay was suggested for accurately diagnosing recent primary human cytomegalovirus infections [65]. Luminol-dependent CL as an indicator of free radical emission is suitable for the detection of the antioxidant status of tissue and to measure the scavenger capacity of the plasma and erythrocytes. A similar method may be useful to evaluate patient states, with or without antioxidant or steroid therapy and to distinguish relapses and inactive periods, as well [66].

CL methods could be better than conventional methods to defect serum antiviral-capsid antigen IgM antibodies to Epstein-Barr virus, and an early diagnosis of infectious mononucleosis could be done [67]. The levels of total IgE in cerebrospinal fluid and serum from peripheral blood were also measured by an automated CL assay, which has implications for specific neurocysticercosis diagnosis [68].

Deitch, SB *et al*. used a cDNA probe, which was prepared from poly(A)+ RNA extracted from simian varicella virus-infected Vero cells at the height of the cytopathic effect (three days after infection), and CL to detect the transcripts corresponding to predicted simian varicella virus open reading frames [69].

APPLICATION OF CL TO EVALUATE THE IMMUNE RESPONSE AGAINST TUMORS

The oxidative stress could be measured by superoxide quantification though lucigenin-amplified CL because some tumors produce more ROS than non-neoplasic tissue. The addition of ROS scavengers to the therapy of gastric tumors has a role in the prevention of gastric carcinoma [70], and it could be applied to evaluate the addition of anti-inflammatory drugs in such cases.

Recently, it has been reported that NO exists in the inner mitochondrial membrane. The mitochondrial utilization of NO involves the production of superoxide anion and H_2O_2 and participates in the modulation of cell proliferation and mitochondrial nitric oxide in the redox state of murine tumors, compared with normal proliferating and quiescent tissues. Decreased oxidative phosphorylation, defective tumoral mitochondrial NO, and low mitochondrial NO-dependent H_2O_2 may used to link persistent tumoral growth to embryonic behavior [71]. This was demonstrated through western blotting analysis using an enhanced CL detection system to detect changes in the oxidative activity.

Additionally, CL was used to determine the effects of eniluracil, which inactivates the dihydropirimidine dehydrogenase activity in human solid tumors, on colorectal tumors and there was important to diminish the resistance in human colorectal cancer [72].

CL ASSAYS TO STUDY ENDOCRINE DISORDERS

CL assays could be applied in studies to determine pulsatile levels of hormones in endocrine states of physiological changes or disease. N. Shah *et al*. evaluated the growth hormone (GH) secretory responses during continuous 24-h intravenous infusion of saline versus the most potent clinically available hexapeptide, GHRP-2 (1 mg/kgh), in estrogen-unreplaced postmenopausal women in a paired, randomized design. The kinetics of the hormonal response was assayed by an ultrasensitive GH CL assay [73].

Human GH and prolactine (PRL) concentrations in the media and cell extracts were determined by a nonisotopic, automatic CL assay performed to compare the activity of hormones analogues on hormone release by cultures of secreting pituitary adenomas. Intra- and inter-assay coefficients of variation for GH and PRL were calculated. Human ACTH, LH, and FSH concentrations were determined by a nonisotopic, automatic CL immunoassay system. Intra- and inter-assay coefficients of variation for ACTH, LH, and FSH were similar. The assays were easy to do, and the time to get the results was considerable shorter compared to conventional and less secure techniques to assess hormone determinations [74].

USE OF CL ASSAYS TO EXPLORE THE INFLAMMATORY RESPONSE OF AIRWAYS

Oxidative stress during sepsis is closely related with multiorgan injuries. A CL technique was used to show that *ex vivo* Vitamin E and simvastatin induced an inhibition of O_2- degree production and that NADPH oxidase inactivation is involved in these phenomenon [75]. This assay could be used to select drugs to stop the O_2- degree production.

Recently, inflammatory pulmonary diseases, particularly severe asthma, have been associated with increased numbers and activities of PMN. Lucigenin-amplified CL appears to be an appropriate technique for measuring alveolar macrophages activity when the proportion of PMN in mixed cell populations is small, and this is a method to explore the increase in PMN activity in cells obtained from asthmatic airways [76].

The inhalation of dusts and environmental toxins is commonly associated to pulmonary diseases. A luminol-dependent CL assay is a new method to study inflammation in both phagocytic cells and lung tissue recovered from dust-exposed animals, which showed potent CL signals when compared with the saline group [77]. CL assays were used to suggest that ginseng treatment leads to an activation of PMNs and modulation of the IgG response to *P. aeruginosa*, enhancing the bacterial clearance and thereby reducing the formation of immune complexes. This resulted in milder lung pathology in a rat model of cystic fibrosis [78]. Finally, even when the functional status of PMNs from patients with adult respiratory distress syndrome has not been previously defined. Neutrophils cause pulmonary damage once circulating PMNs are in an activated state, as demonstrated by CL and are capable to release high levels of inflammatory mediators, such as ROS [79].

CONCLUDING REMARKS

The advantages of CL assays include sensitivity, speed (signal generated in a few seconds), nonhazardous reagents, and simple procedures. The most promising clinical applications are in immunoassays, protein blotting, DNA probe assays, including reporter gene studies and toxicology and

pharmacological tests (e.g. after exposure to antibiotic or agents inmunomodulators as adjuvants and cytotoxic drugs). On the other hand, it can be used consistently to follow patients who have suffered from immunological diseases, to estimate microbial numbers and to assess cellular states [80-82]. The wide variety of applications of CL assays in clinical immunology and the numerous advantages of CL-based methods over other conventional methods to analyze the same endpoints are good arguments for their extensive use in the practice of clinical immunology.

REFERENCES

[1] Campbell, A.K. *Chemiluminescence: Principles and Applications in Biology and Medicine*. Ellis Horwood series in biomedicine. New York. **1988**.

[2] Bronstein, I.; Fortin, J.J.; Voyta, J.C.; Juo, R.R.; Edwards, B.; Olesen, C.E.; Lijam, N.; Kricka, L.J. Chemiluminescent reporter gene assays: sensitive detection of the GUS and SEAP gene products. *Biotechniques*, **1994**, *17*, 176-7.

[3] Barlet, V.; Cohard, M.; Thelu, M.A.; Chaix, M.J.; Baccard, C.; Zarski, J.P.; Seigneurin, J.M.; Seigneurin, J. Quantitative detection of hepatitis B virus DNA in serum using chemiluminescence: comparison with radioactive solution hybridization assay. *J. Virol. Methods*., **1994**, *49*, 141-51.

[4] Alkan, S.; Akdis, C.; Towbin, H. Chemiluminescence and enzyme-linked immuno assays for sensitive detection of human INF-gamma. *J. Immunoassay*., **1994**, *15*, 217-38.

[5] Martin, C.S.; Butler, L.; Bronstein, I. Quantitation of PCR products with chemiluminescence. *Biotechniques*, **1995**, *18*, 908-13.

[6] Martin, C.S.; Wight, P.A.; Dobrestova, A.; Bronstein, I. Dual luminescence-based reporter gene assay for luciferase and beta-galactosidase. *Biotechniques*, **1996**, *21*, 520-4.

[7] Bronstein, I.; Martin, C.S.; Fortin, J.J.; Olesen, C.E.; Voyta, J.C. Chemiluminescence: sensitive detection technology for reporter gene assays. *Clin. Chem*., **1996**, *42*, 1542-6.

[8] Yang, T.T.; Sinai, P.; Kitts, P.A.; Kain, S.R. Quantification of gene expression with a secreted alkaline phosphatase reporter system. *Biotechniques*, **1997**, *23*, 1110-14.

[9] Chen, X.; Zhang, X.E.; Chai, Y.Q.; Hu, W.; Zhang, Z.; Zhang, X.; Cass, A.E.S. DNA optical sensor: a rapid method for the detection of DNA hybridization. *Biosens. Bioelectron*., **1998**, *13*, 451-8.

[10] Prendergast, E.; Proctor, R.A. Simple procedure for measuring neutrophils chemiluminescence. *J. Clin. Microbiol.*, **1981**, *13*, 390-2.

[11] Mehrzad, J.; Duchateau, L.; PyÖrälä, S.; Burvenich, C. Blood and milk neutrophil chemiluminescence and viability in primiparous and pluriparous dairy cows during late pregnancy, around parturition and early lactation. *J. Dairy Sci.*, **2002**, *85*, 3268-76.

[12] Tengler, R.S.; Furukawa, K.; de Weck, A.L.; Maly, F.E. Chemiluminescence of mononuclear cells is enhanced during antigen recognition. *J. Biolumin. Chemilumin*., **1993**, *8*, 159-67.

[13] Lucisano, Y.M.; de-Mello, A.M.; Vasques, E. R.; Mantovani, B. Precipitated immune complexes of IgM induce the generation of reactive oxygen species by rabbit polymorphonuclear leucocytes. *Braz. J. Med. Biol. Res*., **1998**, *31*, 793-7.

[14] Radi, R.; Cosgrove, T.P.; Beckman, J.S.; Freeman, B.A. Peroxynitrite-induced luminol chemiluminescence. *Biochem. J*., **1993**, *290*, 51-7.

[15] Van Dyke, K. In: *Introduction to Cellular Chemiluminescence: Neutrophil, macrophages, and monocyte*. Cellular Chemiluminescence. FL: CRC Press., **1987**, vol. *1*, pp. 3-24.

[16] Towbin, H.; Schmitz, A.; Vanoostrum, J.; Seitz, M.; Dewald, B.; Zingel, O.; Motz, J.; Vosbeck, K.; Rordorf, C. Monoclonal antibody based enzyme-linked and chemiluminescent assays for the human interleukin-1 receptor antagonist. Application to measure hIL-1ra levels in monocyte cultures and synovial fluids. *J. Immunol. Methods*, **1994**, *170*, 125-35.

[17] Carreras, M.; Pargament, C.G.A.; Gatz, S.D.; Poderoso, J.J.; Boveris, A. Kinetics of nitric oxide and hydrogen peroxide production and formation of peroxynitrite during the respiratory burst of human neutrophils. *FEBS Lett*., **1994**, *341*, 65-8.

[18] Kikuchi, K.; Nagano, T.; Hayakawa, H.; Hirata, Y.; Hirobi, M. Detection of nitric oxide production from perfused organ by a luminol-H_2O_2 system. *Anal. Chem*., **1993**, *65*, 794-9.

[19] Wang, J.F.; Komarov, P.; De Groot, H. Luminol chemiluminescence in rat macrophages and granulocytes: The role of NO, O_2-/H_2O_2, and HOCl. *Arch. Biochem. Biophys*., **1993**, *304*, 189-96.

[20] Romaschin, A.D.; Walker, P.M. Endotoxin activity in whole blood by neutrophils chemiluminescence – a novel analytical paradigm. *Clin. Chem*., **2000**, *46*, 1504-6.

[21] Lindsay, M.A.; Keany, C.I.C.; Burden, R.P.; Fletcher, J.; Haynes, A.P. Role of arachidonic acid and its metabolites in the priming of NADPH oxidase in human polymorphonuclear leukocytes by peritoneal dialysis effluent. *Clin. Diagn. Lab. Immunol*., **1998**, *5*, 683-9.

[22] Rodríguez, Orozco, A.R.; Rico, Rosillo, G.; López, Revilla, R. The effect of Cry 1Ac on human monocytes and neutrophil activation. *Allergy Clin. Immunol. Int. J. World Allergy Org*., **2005**, *17*, 64-5.

[23] Kato. M.; Kimura, H.; Motegi, Y.; Tachibana, A.; Minakami, H.; Morikawa, A.; Kita, H. Platelet-activating factor activates two distinct effector pathways in human eosinophils. *J. Immunol*., **2002**, *169*, 5252-9.

[24] Fujisawa, M.; Kojima, K.; Beppu, T.; Futagawa, S.; Kuwahara, K.; Hiramatsu. K. Early diagnosis of postoperative infection: Assessment of whole blood chemiluminescence. *Surg. Today*., **2000**, *30*, 309-18.

[25] Starkebaum, G.; Stevens, D.L.; Henry, C.; Gavin, S.E. Stimulation of human neutrophils chemiluminiscence by soluble immune complexes and antibodies to neutrophils. *J. Lab. Clin. Med*., **1981**, *98*, 280-91.

[26] Doll, N.; Wilson, J.M.R.; Salvaggio, E.J. Inhibition of polymorphonuclear leukocyte chemiluminescence for detection of immune complexes in human sera. *J. Clin. Invest*., **1980**, *66*, 457-64.

[27] Chedraoui-Silva, S.; Mantovani, B. The role of complement in the modulation by fluid-phase IgG of the production of reactive oxygen species by polymorphonuclear leukocytes stimulated with IgG immune complexes. *Braz. J. Med. Biol. Res*., **2003**, *36*, 1665-72.

[28] Badr El-Din, M.K.; Abdel-Salam Madkour, A.; Badr El-Din, O.M.K.; Rashwan, E.A.; Bahr, R.S. Defective monocyte phagocytic function as a possible genetic marker for rheumatic susceptibility. *Eastern Mediterr. Health J*., **1998,** *4*, 36-43.

[29] Mayer, M.; Hermanova, Z. Effect of nitric oxide metabolism upon peripheral blood chemiluminescence and leukocyte and erythrocyte adherence in multiple sclerosis. *Bratisl. Lek. Listy*., **1999**, *100*, 531-6.

[30] Ponugoti, VR.; Maddala, R.; Jhon, F.; Zigler, J.S. Expression of nonphagocytic NADPH oxidase system in the ocular lens. *Mol. Vis*., **2004**, *10*, 112-21.

[31] Cheeson, D.B. The origin of the Chemiluminescence of phagocytosing Neutrophils in patients with primary Sjogren's syndrome. *J. Clin. Invest*., **1977**, *58*, 789-96.

[32] Saeed, A.F.; Castle, E.G. Neutrophil Chemiluminescence during phagocytes is inhibited by abnormally elevated levels of acetoacetate: implications for diabetic susceptibility to infections. *Clin. Diagn. Lab. Immunol*., **1998**, *5*, 740-3.

[33] Mastroianni, C.M.; Lichtner, M.; Mengoni, F.; D'Agostino, C.; Forcina, G.; Santopadre, P.; Vullo, V. Improvement in neutrophils and monocyte function during highly active antiretroviral treatment of HIV-1-infected patients. *AIDS*., **1999**, *13*, 883-90.

[34] Poirier, M.C.; Divi, R.L.; Al-Harthi, L.; Olivero, O.A.; Nguyen, V.; Walker, B.; Landay, A.L.; Walker, V.E.; Charurat, M.; Blattner, W.A. Long-Term Mitochondrial Toxicity in HIV-Uninfected Infants Born to HIV-Infected Mothers. *J. Acquir. Immune Defic Syndr*., **2003**, *33*, 175-83.

[35] Di Renzo, M.; Pasqui, A.L.; Bruni, F.; Saletti, M.; Bova, G.; Chiarion, C.; Carducci, A. Evaluation of some immune functions in a patient affected by common variable immunodeficiency using luminescent techniques. *J. Biolumin. Chemilumin*., **1998**, *12*, 193-7.

[36] McCafferty, A.C.; Cree, I.A. Measurement of cell migration stimulated by interleukin 8: use of ATP chemiluminescence. *Cytokine*, **1994**, *6*, 450-3.

[37] Abruzzo, G.K.; Fromtling, R.A.; Giltinan, D.M.; Effects of bifonazole, fluconazole, itraconazole, and terbinafine on the chemiluminescence response of immune cells. *J. Antimicrob. Chemother*., **1987**, *20*, 61-8.

[38] Khan, H.A. Effect of Fluconazole on phagocytic response of polymorphonuclear leukocytes in a rat model of acute sepsis. *Mediators Inflamm*., **2005**, *24*, 9-15.

[39] Shin, J.E.; Kim, J.M.; Bae, E.A.; Hyun, Y.J.; Kim, D.H. *In vitro* inhibitory effect of flavonoids on growth, infection and vacuolation of Helicobacter pylori. *Planta Med*., **2005**, *71*, 197-201.

[40] Mungall, B.A.; Xu, X.; Klimov, A. Surveillance of influenza isolates for susceptibility to neuraminidase inhibitors during the 2000-2002 influenza seasons. *Virus Res*., **2004**, *103*, 195-7.

[41] Punareewattana, K.; Smith, B.J.; Blaylock, B.L.; Longstreth, J.; Snodgrass, H.L.; Gogal, R.M.; Prater, R.M.; Holladay, S.D. Topical permethrin exposure inhibits antibody production and macrophage function in C57B1/6N mice. *Food Chem. Toxicol*., **2001**, *39*, 133-9.

[42] Sato, K.; Sato, N.; Shimizu, H.; Tsutiya, T.; Takahashi, H.; Kakizaki, S.; Takayama, H.; Takagi, H.; Mori, M. Faropenem enhances superoxide anion production by human neutrophils *in vitro*. *J. Antimicrob. Chemother*., **1999**, *44*, 337-41.

[43] Masihi, K.N.; Brehmer, W.; Azuma, I.; Lange, W.; Muller, S. Stimulation of Chemiluminescence and resistance against aerogenic influenza virus infection by synthetic muramy dipeptide combined with trehalose dimycolate. *Infect. Immun*., **1984**, *43*, 233-7

[44] Hongan, N.A.; Hill, H.R. Enhancement of Neutrophils chemotaxis and alteration of levels of cellular cyclic nucleotides by levamisole. *J Infect Dis*., **1987**, *138*, 437-44.

[45] Braga, P.C.; Salaa, M.T.; Dal Sassoa, M.; Mancinib, L.; Sandrinic, M.C.; Annonic, G. Influence of age on oxidative bursts (chemiluminescence) of polymorphonuclear neutrophils leukocytes. *Gerontology*, **1998**, *44*, 192-7.

[46] Aldinil, R.; Marangoni, A.; Guardigli, M.; Sambri, V.; Giacani, L.; Montagnani, M.; Roda A.; Cevenini. R.; Chemiluminescence detection of reactive oxygen species in isolated Kupffer cells during phagocytosis of *Treponema pallidum*. *Comp. Hepatol*., **2004**, *3*, S41.

[47] Carlyon, J.; Abdel-Latif, A.D.; Pypaert, M.; Lacy, P.; Fikrig, E.; *Anaplasma phagocytophilum* utilizes multiple host evasion mechanisms to thwart NADPH oxidase-mediated killing during neutrophils infection. *Infect. Immun*., **2004**, *72*, 4772-83.

[48] Scaife, H.; Woldehiwet, Z.; Hart, C.A.; Edwards, S.W. Anaplasma phagocytophilum reduces neutrophils apoptosis *in vivo*. *Infect. Immun*., **2003**, *71*, 1995-2001.

[49] Li, Y.; Ma, Q.H.; Dong, Y.G. Infection with Chlamydia pneumoniae increases oxidative stress and accelerates the development of atherosclerosis in C57BL/6J mice. *Zhonghua Xin Xue Guan Bing Za Zhi*., **2005**, *33*, 395-8.

[50] Teneberg, S.; Jurstrand, M.; Karlsson, K.A.; Danielsson, D. Inhibition of nonopsonic *Helicobacter pylori* induced activation of human neutrophils by sialylated oligosaccharides. *Glycobiology*., **2000**, *10*, 1171-81.

[51] Danielsson, D.; Jurstrand, M. Nonopsonic activation of neutrophils by *Helicobacter pilory* is inhibited by Rebamipide. *Dig. Dis. Sci*., **1998**, *43*, 167S-73S.

[52] Hansen, P.S.; Go, M.F.; Varming, K.; Andersen, L.P.; Genta, R.M.; Graham, D.Y.; Nielsen, H. Proinflammatory activation of neutrophils and monocytes by *Helicobacter pilory* in patients with different clinical presentations. *Infect. Immun*., **1999**, *67*, 3171-4.

[53] Matthay, K.K.; Mentzer, W.C.; Wara, D.W.; Preisler, H.K.; Lameris, N.B.; Ammann, A.J. Evaluation of the opsonic requirements for phagocytosis of Streptococcus pneumoniae serotypes VII. XIV and XIX by chemiluminescence assay. *Infect. Immun*., **1981**, *31*, 228-35.

[54] Hofman, P.; Piche, M.; Farahi Far, D.; Negrate, G.L.; Selva, E.; Landraud, L.; Schmid, A.; Boquet, P.; Rossi, B. Increased *Escherichia coli* phagocytosis in neutrophils that have transmigrated across a cultured intestinal epithelium. *Infect. Immun*., 2000, *68*, 449-55.

[55] Mazzulli, T.; Wood, S.; Chua, R.; Walmsley. S. Evaluation of the Digene Hybrid Capture System for detection and quantitation of human cytomegalovirus viremia in human immunodeficiency virus-infected patients. *J. Clin. Microbiol*., **1996**, *34*, 2959-62.

[56] Barbour, A.G.; Allred, C.D.; Solberg, C.O.; Hill, H.R. Chemiluminescence by polymorphonuclear leukocytes from patients with active bacterial infection. *J. Infect. Dis*., **1980**, *141*, 14-26.

[57] Solberg, C.O.; Kaiager, T.; Hill, H.R.; Glette, J. Polymorphonuclear leukocyte function in bacterial and viral infections. *Scand. J. Infect. Dis*., **1982**, *14*, ll-8.

[58] Shah, S.V.; Wallin, J.D.; Eilen, S.D. Chemiluminescence and superoxide anion production by leukocyte from diabetic patients. *J. Clin. Endocrinal. Metab*., **1983**, *57*, 402-9.

[59] Huang, H.H.; Ya, H.C.; Han, C.L.; Yu, F.C.; Kao, W.Y.; Chen, W.T. Association of *in vitro* oxidative stress, serum ferritin concentration and C-reactive protein in febrile emergency room patients. *Clin. Invest. Med*., **2005**, *28*, 48-54.

[60] Salo, M.; Perttila, J.; Lehtonen, O.P. Granulocyte Chemiluminescence in patients with postoperative infections. *Arch. Surg*., **1988**, *123*, 120-34.

[61] Khan, H.A. Zymosan-induced luminal-dependent Chemiluminescence response of circulating and extravasated leukocytes in experimental sepsis. *Mediators Inflamm*., **2004**, 13, 123-5.

[62] Terekhina, N.A.; Petrovich, L.A. Activity of antioxidant enzymes and peripheral blood lymphocyte Chemiluminescence in herpes simplex virus infection of the eye. *Vopr Med Khim.*, **1992**, *38*, 62-3.

[63] Bondestam, M.; Hakansson, L.; Foucard, T.; Venge, P. Defects in polymorphonuclear Neutrophils function and susceptibility to infection in children. *Scand. J. Clin*., **1986**, *46*, 685-94.

[64] Amaize, E.A. A raped and sensitive Chemiluminescence assay for evaluation of opsonic activity of *Haemophilus influenzae* type *b*-specific antibodies. *Mol. Hum. Reprod*., **2000**, *6*, 883-91.

[65] Revello, M.G.; Gorini, G.; Gerna, G. Clinical evaluation of a chemiluminescence immunoassay for determination of immunoglobulin g avidity to human cytomegalovirus. *Clin. Diagn. Lab. Immunol*., **2004**, *11*, 801-5.

[66] Blazovies, A.; Kovacs, A.; Lugasi, A.; Hagymasi, K.; Biro, L.; Free, J. Antioxidant defense in erythrocytes and plasma of patients with active and quiescent crohn disease and ulcerative colitis: A Chemiluminescent study. *Clin. Chem*., **1999**, *45*, 895-6.

[67] Feug, Z.; LiZ, S.B.; Xu, G.; Xia, T. Serological diagnosis of infectious mononucleosis by chemiluminescent immunoassay using capsid antigen p8 of Epstein-Barr virus. *Clin. Chim. Acta*, **2005**, *354*, 77-82.

[68] Casagranda, E.B.; Adelaide, J.V.; dos Ramos, L.M.; Antonio, J.L. Total IgE detection in paired cerebrospinal fluid and serum sample from patients with neurocysticercosis. *Rev. Inst. Med. Trap*., **2000**, *42*, 67-70.

[69] Deitch, S.B.; Gilden, D.H.; Wellish, M.; Smith, J.; Cohrs, R.J.; Mahalingam, R. Array analysis of simian varicella virus gene transcription in productively infected cells in tissue culture. *J. Virol*., **2005**, *79*, 5315-25.

[70] OIiveira, C.; Kassab, P.; Lopasso, F.P.; Souza, H.P.; Zewski, M.J.; Laurindo, F.M.R.; Iriya, K.; Laudanna, A.A. Protective effect of ascorbic acid in experimental gastric cancer: reduction of oxidative stress. *World J. Gastroenterol*., **2003**, *9*, 446-8

[71] Galli, S.; Labato, M.I.; Bal de Kier, J.E.; Carreras, M.C.; Poderoso, J.J. Decreased mitochondrial nitric oxide synthase activity and hydrogen peroxide relate persistent tumor proliferation to embryonic behavior. *Cancer Res*., **2003**, *63*, 6370-7.

[72] Ahmed, F.Y.; Johnston, J.S.; Cassidy, J.; O'Kelly, T.; Vine, N.; Murria, I.G.; Gennip, H.A.; Knight, S.; McLeod, L.H. Eniluracil treatment completely inactivates dihydropyrimidine dehydrogenase in colorectal tumors. *J. Clin. Oncol*., **1999**, *17*, 2439-45.

[73] Shah, N.; Evans, W.S.; Bowers, C.Y.; Veldhuis, J.D. Tripartite Neuroendocrine activation of the human growth hormone (GH) axis in women by continuous 24-hour GH-releasing peptide infusion: Pulsatile, Entropic, and Nyctohemeral Mechanisms. *J. Clin. Endocrinol. Metab*., **1999**, *84*, 2140–50.

[74] Hofland, L.J.; Hoek, J.V.D.; Koetsveld, P.M.V.; Herder, W.W.; Waaijers, M.; Sprij-Mooij, D.; Bruns, C,; Weckbecker, G.; Feelders, R.; Lely, A.J.V.D.; Beckers, A.; Lamberts, S.W.J. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas *in vitro*. *J. Clin. Endocrinol. Metab*., **2004**, *89*, 1577–85.

[75] Durant, R.; Klouche, K.; Delbose, S.; Morena, M.; Amigues, L.; Beraud, J.J.; Canaud, B.; Cristol, J.P. Superoxide anion overproduction in sepsis: effects of vitamin E and simvastatin. *Shock*., **2004**, *22*, 34-9.

[76] Ward, C.; Kelly, C.A.; Stenton, C.S.; Duddridge, M.; Hendrick, D.J.; Walters, E.H. The relative contribution of broncho alveolar

macrophages and Neutrophils to lucigenin-and luminol-amplified Chemiluminescence. *Eur. Respir. J*., **1990**, *3*, 1008-14.

[77] Antonini, J.M.; Van Dyke, K.; Ye, Z.; DiMatteo, M.; Reasor, M.J. Introduction of luminol-dependent chemiluminescence as a method to study silica inflammation in the tissue and phagocytic cells of rat lung. *Environ. Health Perspect*., **1994**, *102*, 37S-42S.

[78] Zhijun, S.; Kharazmi, A.; Wu, H.; Faber, V.; Moser, C.; Krogh, H.J.; Rygaard, J.; Hoiby, N. Effects of ginseng treatment on Neutrophil Chemiluminescence and immunoglobulin *G* subclasses in a rat model of chronic *Pseudomonas aeruginosa* pneumonia. *Clin. Diagn. Lab. Immunol*., **1998**, *5*, 882-7.

[79] Zimmermau, G.A.; Renzetti, A.D.; Hill, H.R. Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome. Evidence for activated Neutrophils in the pulmonary circulation. *Am. Rev. Respire. Dis*., **1983**, *127*, 290-300.

[80] Kricka, L.J. Chemiluminescent and bioluminescent techniques. *Clin. Chem*., *1991*, 37, 1472-81.

[81] Champiat, D.; Roux, A.; Lhomme, O.; Nosenzo, G. Biochemiluminescence and biomedical applications. *Cell. Biol. Toxicol*., **1994**, *10*, 345-51.

[82] Nicolas, J.C.; Boussioux, A.M.; Joyeux, A.; Balaguer, P.; Térouanne, B.; Roux, S.; Gagne, D.; Pons, M. Applications of luminescence reactions in life science. *C. R. Seances Soc. Biol. Fil*., **1995**, *189*, 740-53.

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