**CHINESE JOURNAL OF ANALYTICAL CHEMISTRY**
Volume 43, Issue 6, June 2015
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

**Cite this article as:** Chin J Anal Chem, 2015, 43(6), 929–938.

**REVIEW**

# Advances and Applications of Chemiluminescence Immunoassay in Clinical Diagnosis and Foods Safety

**XIAO Qin[1,2], LIN Jin-Ming[2,*]**

[1] College of Ocean, Hebei Agricultural University, Qinhuangdao 066003, China
[2] Department of Chemistry, Beijing Key Laboratory of Microanalytical Methods and Instrumentation, Tsinghua University, Beijing 100084, China

**Abstract:** Chemiluminescence immunoassay (CLIA) has been widely used in different fields including clinical diagnosis, food safety, and environmental assessment for its high sensitivity, selectivity, and rapid and simple analysis. The development of novel CLIA with high sensitivity, specificity and throughput has recently become a research focus and trend. In this review, we introduced some new CLIA methods and their coupling techniques, as well as the applications in clinical diagnosis and foods safety since 2011. The development of CLIA in the future was also prospected.

**Key Words:** Chemiluminescence immunoassay; Magnetic particles; Gold nanoparticles; Resonance energy transfer; Tumor marker; Review

## 1 Introduction

Chemiluminescence immunoassay (CLIA), a technique of chemiluminescence (CL) combined with immunoassay (IA), is extensively applied in various fields including clinical diagnosis, food safety, and environmental assessment for its high sensitivity, good specificity, rapid analysis and simple operation.

CLIA has been developed for more than 40 years since it was proposed by Tsuji *et al* in 1977. Although CLIA is a developed system nowadays, the traditional CLIA could not meet the requirements for the analyses of complex samples with complex matrix or low abundance. Thus, the development of novel CLIAs with high performance, such as high sensitivity, specificity and throughput, becomes a research focus. In this review, the research literatures of CLIA were summarized since 2011. The majority of this paper introduced the research progress in CLIA methods, their coupling with relevant techniques, as well as their applications in clinical diagnosis and food safety. The future development of CLIA was also discussed.

## 2 Novel methods of CLIA

Although traditional CLIA performs well for conventional tests, it has some weaknesses such as low specificity or low signal to noise ratio (SNR) for the complex matrix and low abundance samples. In addition, the traditional CLIA would not meet the demands for high sensitivity analyses due to its low energy transfer efficiency from the chemical energy to light energy. Consequently, it is the primary ways to improve the specificity and sensitivity by reducing background interference and enhancing signal intensity. Nowadays, the breakthroughs on improving separation efficiency and sensitivity through the use of magnetic particles (MPs) or gold nanoparticles (AuNPs) based CLIA were reported.

### 2.1 Magnetic particles based CLIA

The separation of antigen-antibody immune complex is usually a complicated and time-consuming procedure in immunoassay, which is a primary cause of low SNR in CLIA. Magnetic particles (MPs), as a solid phase of reaction, are

Received 30 December 2014; accepted 2 March 2015
* Corresponding author. Email: jmlin@mail.tsinghua.edu.cn
This work was supported by the National Natural Science Foundation of China (No. 81373373) and the Specialized Research Fund for the Doctoral Program of Higher Education, China (No.20110002110052)

DOI: 10.1016/S1872-2040(15)60831-3

utilized in CLIA as a promising way to improve this process. MPs coated with antibodies can capture the antigens in immunoreactions to form immune complex, and under magnetic field, the obtained immune complex can be quickly separated from the original solution. The separation step can eliminate the matrix effect and reduce the background interference. This technique was successfully applied to practical sample analysis. Table 1 lists the examples of magnetic particles as solid phase carriers in CLIA in the past 4 years.

Most of CLIAs are usually designed to a sandwich type detection method. The antibody immobilized on the surface of MPs can capture the target analyte (antigen) and react with enzyme-labeled antibodies to form an immune complex by a double antibodies sandwich reaction. The obtained immune complex is separated and removed from the unwanted materials by using an external magnetic field. The CL signal is measured after adding luminescent substrate. Liu *et al*[10] reported a MPs based CLIA for the determination of prostate-specific antigen (PSA) in human serum. In this method, a sandwiched reaction was formed between the anti-PSA antibody coated on 2.8 μm MPs, PSA sample and alkaline phosphatase (ALP) labeled anti-PSA antibody. The immune complex was separated by using an external magnet. As mentioned above, the CL signal was produced and measured after adding the chemiluminescent substrate. Compared with the commercial microplate CLIA kit for PSA detection, the introduction of MPs not only reduced the amounts of reagent used by half, but also remarkably shortened analysis time. More importantly, it could reduce the background noise and enhance the sensitivity in CLIA.

The introduction of MPs promoted the development of CLIA technique. A great deal of scientific researches appeared about the influence of MPs physical properties on CLIA. As we can see from the Table 1, the size of MPs is usually in the range from nanometers to micrometers. The effect of MP size on CLIA was reported. Zhang *et al*[3] analyzed the influence of different sizes of MPs on CLIA. The result showed that 100 nm MPs were better than 2 μm MPs in both sensitivity and detection time. Although Dai *et al*[12] showed that 200 nm MPs had a higher detection signal than that of 1 μm MPs, however, the 1 μm MPs had a weaker nonspecific adsorption, namely that the background value was lower, and could accumulate rapidly under the influence of a magnetic field. So the larger particle size (1 μm MPs) was more suitable for an automated CLIA system. As can be seen from the two above reports, the results were consistent with regard to sensitivity. That is to say, the smaller size MPs is better than the larger one as far as the sensitivity is concerned. As for the assay time, Zhang's results showed that small size MPs shortened the immunoreaction time by 30 min. The result of Dai's group was that with regard to separation time, the large size MPs was faster than the small ones by 80 s in separation time under the additional magnetic field. As a comprehensive summary, it is seen that small size MPs have the superior immunoassay time. Yet it needs to be pointed out that for an equal number of MPs, 1 μm MPs have a lower nonspecific adsorption which is attributed to its relatively small specific surface area.

The surface modifications of MPs are also of great importance in MPs based CLIA. Aside from the direct modification by antibody[10], the amplified systems e.g. streptavidin-biotin[3,11] or fluorescein isothiocyanate (FITC)-anti FITC[6,7,9] were also used for the modification of MPs. Additionally, Ma *et al*[13] reported a novel modification manner in which MPs were first modified with polyethyleneeimine (PEI) by acylamide bond, followed by immobilizing of the antibody onto PEI with glutaradehyde as linkage. PEI enlarged the distance between MPs and the antibody, and reduced the steric hindrance in immune reactions. The result showed that the CL signal was enhanced up to 4-fold compared with that using MPs without PEI modification.

Table 1 Examples of magnetic particles as solid phases in CLIA

| Analytes | Magnetic particles size | Linear range | Detection limit | Reference |
|---|---|---|---|---|
| Caspase-3 | 1 μm | 1.0–600 ng $mL^{-1}$ | 0.3 ng $mL^{-1}$ | [1] |
| Carcinoembryonic antigen (CEA) | 10 nm | 1.0–80 ng $mL^{-1}$ | 0.25 ng $mL^{-1}$ | [2] |
| α-Fetoprotein (AFP) | | 1.0–75 ng $mL^{-1}$ | 0.5 ng $mL^{-1}$ | |
| Glypican-3 (GPC3) | 100 nm | 0–2500 ng $mL^{-1}$ | 0.38 ng $mL^{-1}$ | [3] |
| Hydrocortisone (HCOR) | 30 nm | 9.0–900 nM | 3.9 nM | [4] |
| Corticosterone (COR) | | 9.0–900 nM | 4.4 nM | |
| Digoxin (DIG) | | 8.0–800 nM | 3.6 nM | |
| Testosterone | | 9.0–900 nM | 4.9 nM | |
| Estriol (E3) | | 9.0–900 nM | 3.9 nM | |
| Microcystin-LR (MC-LR) | 150 nm | 0.02–200 mg $L^{-1}$ | 0.006 mg $L^{-1}$ | [5] |
| Neuron specific enolase (NSE) | 1.0 μm | 0.0–300 ng $mL^{-1}$ | <0.2 ng $mL^{-1}$ | [6] |
| Protein S100 B | 1−2 μm | 0.0–25 ng $mL^{-1}$ | 0.02 ng $mL^{-1}$ | [7] |
| Protein S100 B | 1.5 μm | 0.02–1 ng $mL^{-1}$ | 0.005 ng $mL^{-1}$ | [8] |
| Neuron specific enolase (NSE) | | 1–20 ng $mL^{-1}$ | 0.2 ng $mL^{-1}$ | |
| Squamous cell carcinoma antigen (SCC) | 1−2μm | 0–20 ng $mL^{-1}$ | 0.02 ng $mL^{-1}$ | [9] |
| Prostate specific antigen (PSA) | 2.8 μm | 0.1–30 ng $mL^{-1}$ | 0.1 ng $mL^{-1}$ | [10] |
| Carcinoembryonic antigen (CEA) | 1 μm | 0–50 ng $mL^{-1}$ | 5.0 pg $mL^{-1}$ | [11] |