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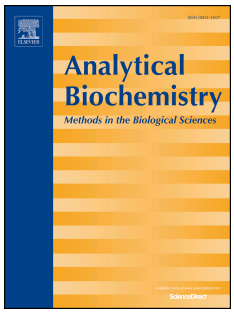

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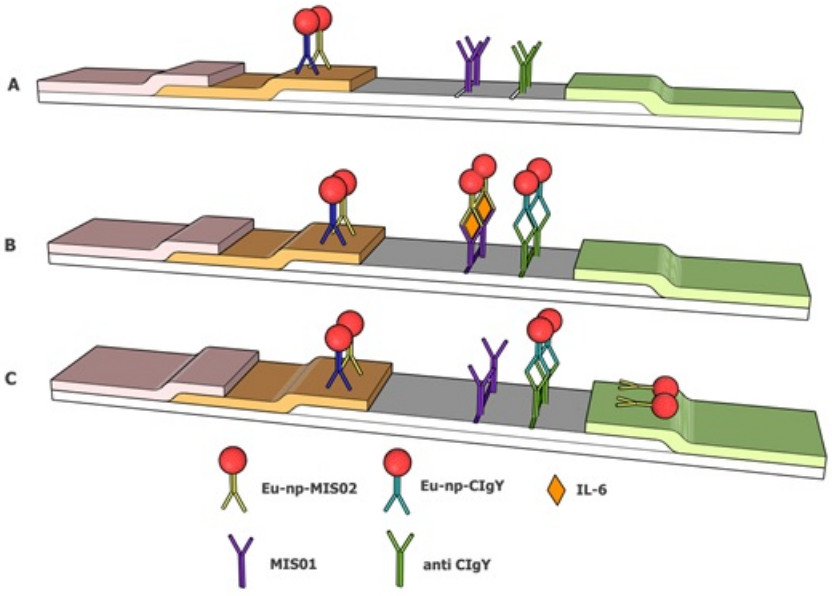

Rapid and sensitive detection of interleukin-6 in serum via time-resolved lateral flow immunoassay

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Abstract: Interleukin 6 (IL-6) is an interleukin that acts as both a proinflammatory and anti-inflammatory cytokine. It can be used as a potential diagnostic biomarker for sepsis. The aim of this study was to establish an easy-to-use detection kit for rapid, quantitative and on-site detection of IL-6. To develop the new IL-6 quantitative detecting kit, a double-antibody sandwich immunofluorescent assay was employed based on europium nanoparticles (Eu-np) combined with lateral flow immunoassay (LFIA). The performance of the new developed kit was evaluated in the aspects of parallel analysis, linearity, sensitivity, precision, accuracy, specificity and clinical sample analysis. Two-hundred and fourteen serum samples were used to carry out the clinical sample analysis. The new IL-6 quantitative detecting kit exhibited a wide linear range (2-500 pg/mL) and a good sensitivity (0.37 pg/mL). The intra-assay coefficient of variation (CV) and the inter-assay CV were 5.92%–8.87% and 7.59%–9.04%, respectively. The recovery rates ranged from 102% to 106%. Furthermore, a high correlation ($n = 214$, $r = 0.9756$, $p < 0.01$) was obtained when compared with SIEMENS CLIA IL-6 kit. Thus, the new quantitative method for detecting IL-6 has been successfully established. The results indicated that the newly-developed strip based on Eu-np combined with LFIA was a facile, fast, highly sensitive, low-cost, reliable biosensor and suitable for rapid and point-of-care test (POCT) for IL-6 in serum.

Keywords: Lateral flow immunoassay; Interleukin-6; Time-resolved; Point-of-care

1 Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. A tentative extrapolation of data from high-income countries suggests that 31.5 million cases of sepsis and 19.4 million cases of severe sepsis occur globally each year, with potentially 5.3 million deaths annually [2]. The cause of so many deaths and so high mortality is due to diagnostic difficulties [3]. Differentiating sepsis from noninfectious systemic inflammatory response syndrome (SIRS) is not straightforward and presents a common dilemma for the intensive care physician. Diagnostic uncertainty (to treat as sepsis or noninfectious SIRS) may delay the initiation of lifesaving standard therapies [4]. Each hour of delay in antimicrobial administration over the ensuing 6 h was associated with an average decrease in

survival of 7.6% [5]. So it is important to recognize it early, so that supportive measures which have been shown to be successful may be implemented as soon as possible [6]. Positive blood culture might be the only means to confirm the diagnosis of sepsis. However, it usually takes 24 to 48 hours to attain culture result with subsequent delay in the diagnosis and treatment of sepsis [7]. In comparison, serum IL-6 rises within a few minutes after a stimulus (infection, trauma or any other inflammation), with a half-life of about an hour [8]. The measurement of IL-6 in patients with suspected sepsis could increase the precision of diagnosis and improve patient management, so it can be used as a potential diagnostic biomarker for sepsis, especially in the diagnosis of neonatal sepsis. Therefore, IL-6 is an inflammatory biomarker with mortality prognostic value and serum concentrations of IL-6 have been correlated with the mortality of septic patients [11,12].

IL-6 is a multifunctional protein that belongs to the glycoprotein-130 (gp130) cytokine family [13]. Human interleukin-6 (IL-6) is a 26 kDa glycoprotein consisting of 184 amino acids, and it was originally identified as a regulator of B-cell differentiation in 1986. IL-6 can be synthesized by a wide variety of cells, including monocytes, macrophages, lymphocytes, fibroblasts, keratinocytes, endothelial cells, and some tumor cells [14]. IL-6 is a pleiotropic cytokine with a wide range of biological activities in immune regulation, hematopoiesis, inflammation and oncogenesis [15]. In healthy adults, IL-6 concentrations in the plasma are <10 pg/mL [16]. Traditionally, IL-6 concentration is measured by enzyme-linked immunosorbent assay (ELISA) [17], chemiluminescent immunoassay (CLIA) [18], electrochemical sensor electrochemical sensor [19] and so on (Table 1). Most of them have a good sensitivity and a wide linear range. However, there are some obvious drawbacks including large equipments, complex operation, time consuming and expensive. As a comparison, lateral flow immunoassays (LFIA), also known as immunochromatographic assays (ICA) are the technology which permit the performance of fast, simple, facile, economic and on-site diagnostics with good robustness, specificity, sensitivity and low limits of detection [20,21].

Table 1

A comparison of assays reported for detection of interleukin-6 (IL-6).

Assay	Sensitivity	Linear range
ELISA[17]	1 pg/mL	1 pg/mL-1 μg/mL
Chemiluminescence immunoassay [18]	0.5 pg/mL	4.0-625.0 pg/mL
Electrochemical sensor [19]	1 pg/mL	0.002-20 ng/mL
Surface plasmon resonance (SPR) biosensors [22]	0.78 ng/mL	0.78–12.5 ng/mL
SERS-based lateral flow assay biosensor [23]	1 pg/mL in PBS, 5 pg/mL in unprocessed whole blood	0.01-400 ng/mL

Electrochemical impedance sensor [24]	0.01 fg/mL	0.01-100 fg/mL
Liquid-gated field-effect transistor (FET) sensors [25]	1.37 pg/mL	-
Graphene Oxide-based amperometric sensor [26]	4.7 pg/mL	4.7-300 pg/mL
Electrochemical immunosensor [27]	0.059 pg mL	0.1-100000 pg/ mL
Electrochemical magnetoimmunosensor [28]	0.39 pg/mL	1.75-500 pg/mL
Photoelectrochemical immunoassay [29]	0.38 pg/mL	1.0 pg/mL-100 ng/mL
Label-free electrochemical aptasensor [30]	0.33 pg mL	1 pg/mL-15 μg/mL
Magnetic colorimetric immunoassay [31]	0.04 pg mL	0.0001-10 ng/mL
Combined electrochemiluminescent (ECL) and electrochemical (EC) immunoassay [32]	0.32 fg/mL for EC, 3.5 ag/mL for ECL	10 fg/mL-90 ng/mL for EC, 10 ag/mL-90 ng/mL for ECL

LFIA emerged at the end of the 1960s for the first time, being used of monitoring of serum proteins. The first homemade LFIA was performed in 1976 to detect human chorionic gonadotropin (hCG) in urine [33]. As yet, gold nanoparticles are the most widely used and are still popular in LFIA [34]. In recent years, many emerging labels have been used for LFIA, such as carbon nanoparticles [35], latex beads [36], up□ converting phosphor particles [37], quantum dot [38], fluorescent europium (III) chelate doped nanoparticle (Eu-np) [39], magnetic nanolabels [40]. Among them, Eu-np have unique luminescence properties, such as long fluorescence lifetimes, narrow emission spectra, large stokes shifts and extremely sharp emission profile with the full width at half-maximum of about 10 nm, and emission from atomic states, all of which contribute to high assay sensitivity and accuracy [33,41]. Based on the above excellent features, we select Eu-np as labels to combine with LFIA to establish a biosensor to detect the concentration of IL-6 in serum. Therefore, we find there are no biosensors based on LFIA for the detection of IL-6 except an “SERS-based lateral flow assay biosensor” [23,42].

2 Materials and Methods

2.1 Materials and Reagents

IL-6 monoclonal antibody (MIS01, MIS02), chicken IgY (CIgY), goat anti-chicken IgY and antigen (IS01) were obtained from YBX Biotechnology Co., Ltd. (Hangzhou, Zhejiang, China). Nitrocellulose (NC) membrane was purchased from Millipore (Bedford, MA, USA). Sample pad, conjugate pad, and absorbent pad were all

purchased from Kinbio Tech.Co.,Ltd. (Shanghai, China). Europium nanoparticles (Eu-np) (200 nm) were acquired from Bangs Laboratories Inc. (Fishers, IN, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), bovine serum albumin (BSA), casein, trehalose, PEG-20000, sodium chloride, boric acid, sodium tetraborate, Tris-HCl, Tween-20, Tris, NaCl, KH_2PO_4, Na_2HPO_4, KCl, methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparing ultrapure water.

2.2 Buffer Solutions

The buffer solutions were as follows: sample pad treatment buffer (50 mM Tris, 3.6 mM EDTA-Na_2, 2% trehalose, 1% BSA, 0.6% Tween-20, pH 8.6); conjugate pad treatment buffer (2 mM boric acid , 3% trehalose, 1% BSA.0.5% casein and 0.01% PEG-20000, pH 9.0); blocking buffer (50 mM Tris-HCl, 1% BSA, 0.05% casein, 0.05% Tween-20, pH 8.0); binding buffer (50 mM borate buffer, pH 7.5); labeling antibody dilution buffer (50 mM Tris-HCl, 0.5% PVA, 0.5% casein, and 3% trehalose, pH 8.0); activating buffer (50 mM borate buffer, pH 7.0); sample dilution buffer (137 mM NaCl , 2mM KH_2PO_4, 8mM Na_2HPO_4, 2.6mM KCl, 0.1% Tween-20, 0.1% BSA, pH 7.4); storage buffer (50 mM Tris-HCl, 0.5% BSA, 0.1% casein, 3% trehalose, 0.05% Tween-20, pH 8.0) and coating buffer (137 mM NaCl, 2 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.6 mM KCl, 3% trehalose, 5% methanol, pH 7.4).

2.3 Experiment Instruments

The CTS3000 strip cutting machine, ZQ4000 high speed cutting machine, HM8000 dispense workstation and XYZ HM3030 dispense workstation were purchased from Kinbio Tech. Co., Ltd. (Shanghai, China). The portable fluorescence strip reader was purchased from Microdetection Tech. Co., Ltd (Nanjing, Jiangsu, China). The probe sonicator (JY92-IIN) was obtained from Ningbo Scientz Biotechnology Co., Ltd. (Ningbo, Zhejiang, China).

2.4 Preparation of Eu-np Coupled with Antibody

To prepare the Eu-np-antibody conjugates (Eu-np-Ab), 100 µL of 200 nm Eu-np were activated for 15 min in 900µL of activating buffer containing 48 µL EDC (10 mg/mL) with shaking firstly. After that, the activated Eu-np were then washed once with 1 mL of binding buffer, and the supernatant discarded after centrifugation at 14,000× g for 15 min at 4 □. Then the activated Eu-np were resuspended in 1 mL of binding buffer by sonication. Four hundred micrograms of antibody (anti-IL-6 MIS02 or CIgY) was added. The coupling reaction was performed for 2.5 h while gently blending. After removing the uncoupled antibody by centrifugation at 14,000× g for 15 min at 4 □, 1 mL of blocking buffer was added to the mixture, shaking for 1 h.

Subsequently, the conjugate solution was resuspended by sonication. Finally, the precipitate (Eu-np-MIS02 or Eu-np-CIgY) was dissolved in 1 mL of storage buffer and stored at 4 □.

2.5 Preparation of the Eu-np Test Strip

The Eu-np test strip was composed of five constituents: a sample pad, a conjugate pad, a NC membrane, an absorbent pad and a PVC pad. Sample pad and the conjugate pad were immersed in sample pad treatment buffer and conjugate pad treatment buffer for 0.5 h at room temperature respectively, and then all were dried at 37 □ for 24 h. The Eu-np-CIgY conjugates and the Eu-np-MIS02 conjugates were mixed together, and diluted 10-folds and 2-folds in labeling antibody dilution buffer respectively. Then the mixture was dispensed onto a pretreated conjugate pad by XYZ HM3030 dispense workstation at the speed of 4 μL/mm, and then dried at 37 □ for 24 h. MIS01 and anti-CIgY were both diluted to 1 mg/mL with the coating buffer. MIS01 (1 mg/mL) and anti-CIgY (1 mg/mL) was sprayed onto the NC membrane as the test line (T) and the control line (C) respectively by the HM8000 dispense workstation, and then dried at 37 □ for 24 h. The PVC pad was then cut into 4 mm wide strips by ZQ4000 high speed cutting machine. The prepared test strips were stored in a drying oven.

2.6 Serum Samples

A total of 214 serum samples were collected from patients at Xinqiao Hospital, Army Medical University (Chongqing, China), including 120 males and 94 females (ages from 13-93 years old). All samples were stored at -20 □ until use. The study was reviewed and approved by the clinical research ethics committee of the Xinqiao Hospital, Army Medical University.

2.7 Sample Detection and Analysis

Initially, 70 μL of sample (standard or serum) was added onto the sample pad. Fifteen minutes later, the results of fluorescence intensity on the T line (H_T) and the C line (H_C) were observed by the reader. The series of reference standards (0, 2, 5, 10, 20, 50, 100, 200, 500, 600, 700 and 1000 pg/mL) were set for standard curve making. Quantitative detection was achieved by recording the fluorescence signal intensity of H_T and H_C and based on the H_T/H_C ratio to effectively eliminate strip-to-strip variation and matrix effects [41].

2.8 Statistical Analysis

The standard curve, sample means and standard deviations (SD) were determined by using OriginPro 8.6 (OriginLab) and Microsoft Excel (version 2016; Analyse-it Ltd, Leeds, UK). Signal linearity and correlations were checked using Pearson's linear regression equation. Linear regression and Pearson's correlation coefficient were utilized to analyze and compare the serum samples measured using the IL-6 LFIA test strips and SIEMENS CLIA kit for IL-6 on SPSS 25.0 (Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3 Results

3.1 Principle of the method

In order to quantify IL-6, we used europium nanoparticles (Eu-np) as label based on LFIA, which were based on a conventional sandwich immunoassay. As shown in Fig. 1A, the strip was composed of sample pad, conjugate pad, NC membrane, absorbent pad, and PVC pad: sample pad was loaded with the prepared sample; conjugate pad was loaded with the Eu-np-MIS02 and Eu-np-CIgY; NC membrane was coated with MIS01 and anti-CIgY; absorbent pad was functioned as a liquid sink, and PVC pad provided support for all the parts mentioned above. As shown in Fig. 1B, when the sample containing IL-6 was added to the sample well, the analytes migrated to the conjugate pad to combine with Eu-np-MIS02 and formed the complexes Eu-np-MIS02-IL-6. After the complexes reached the T line, they were captured by MIS01 coated on the NC membrane and formed Eu-np-MIS02-IL-6-MIS01 sandwich complexes. In this process, the labeled microparticles were immobilized at the detection line. The Eu-np-CIgY complexes continued moving along the membrane and were captured in the control region by the anti-CIgY to form Eu-np-CIgY-anti-CIgY complexes, which served as the internal control. Lastly, surplus fluorescent microsphere complexes migrated into the absorbent pad. When there was no IL-6 in the sample, the T line had no Eu-np-MIS02-IL-6-MIS01 sandwich complexes, only the C line had Eu-np-CIgY-anti-CIgY (Fig. 1C). After reaction completed, the test strip was analyzed with the portable fluorescence strip reader by measuring the fluorescence intensities of the test line (H_T) and the control line (H_C). On the basis of the above, the concentration of IL-6 in the samples would be directly proportional to the ratio of H_T/H_C.

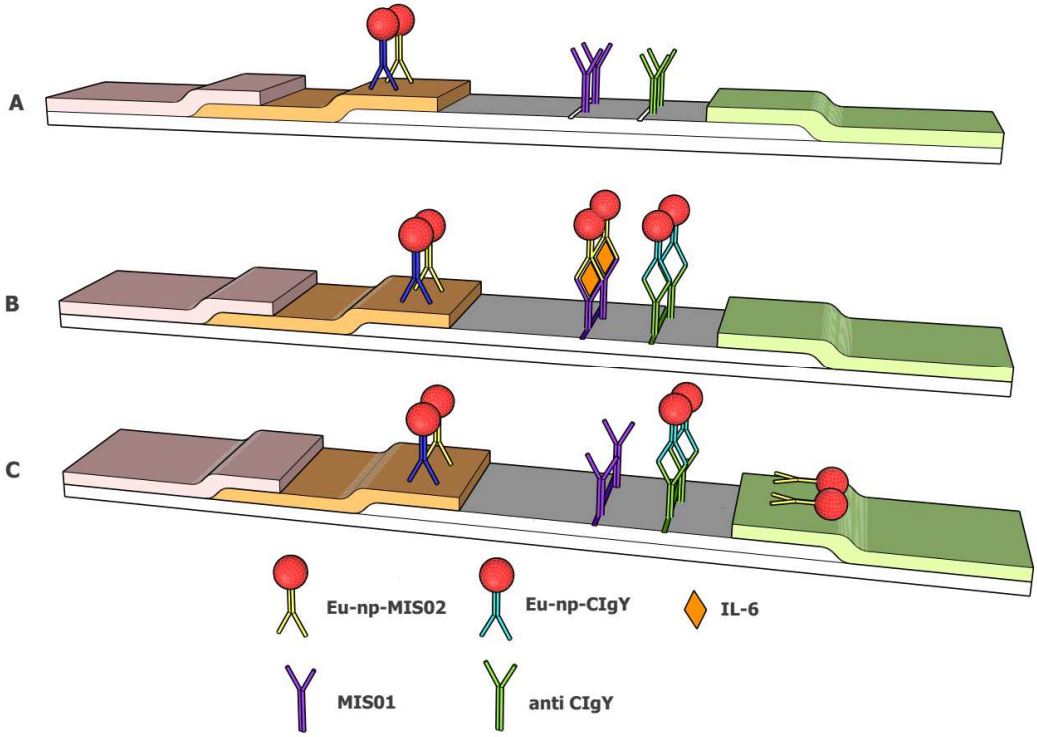

Fig. 1. Schematic illustration of the fluorescence lateral flow assay. (A) Components of IL-6 test strips. (B) Positive sample was added to the sample pad. (C) Negative sample was added to the sample pad.

3.2 Optimization of the immunoreaction time

It is well known that immunoreaction time is a key element which influences fluorescence intensity development in the strip test. In this case, we use four different concentrations of IL-6 standard including 0 pg/mL, 5 pg/mL, 20 pg/mL and 50 pg/mL, and each concentration was performed in triplicate. The results are shown in Fig. 2. At the three concentrations of 5 pg/mL, 20 pg/mL and 50 pg/mL, the H_T/H_C ratio increased rapidly in the first 15 min, and then changed only slowly after 15 min. At the concentration of 0 pg/mL, as T line is closer to sample well than C line, the fluorescence of T line became steady faster than C line. The H_T/H_C ratio declined from sixth minute and became steady at twelfth minute. In consideration of shortening the turnaround time while gaining the best signal intensity, an immunoreaction time of 15 min was chosen as optimum for this system. Finally, we selected 15 min as the most suitable incubation time for further studies.

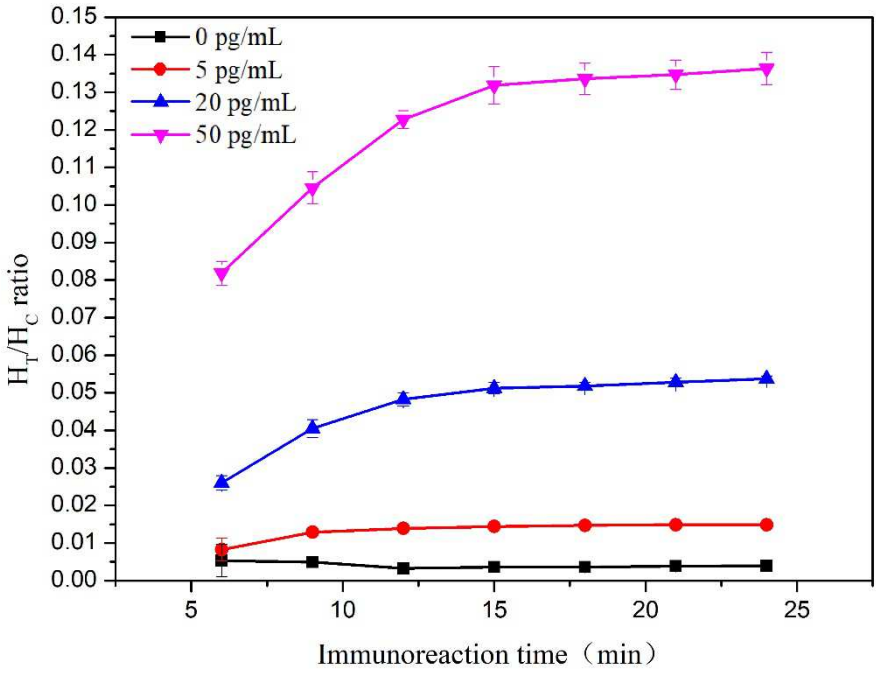

Fig. 2. Optimization of the immunoreaction time.

3.3 Parallel analysis

Parallel analysis was performed to demonstrate the reliability of the assay based on serial dilution. Three serum samples were diluted over the range 1/2 to 1/32 with sample dilution buffer and then analyzed. Each test was conducted in triplicate. As shown in Fig. 3, the three samples exhibited good linearity, the R^2 of the three samples are 0.9945, 0.9953 and 0.9950 respectively, which manifested little variation in the observed concentrations after correcting for sample dilution. On this basis, the proposed method was decidedly suitable for conducting survey analysis.

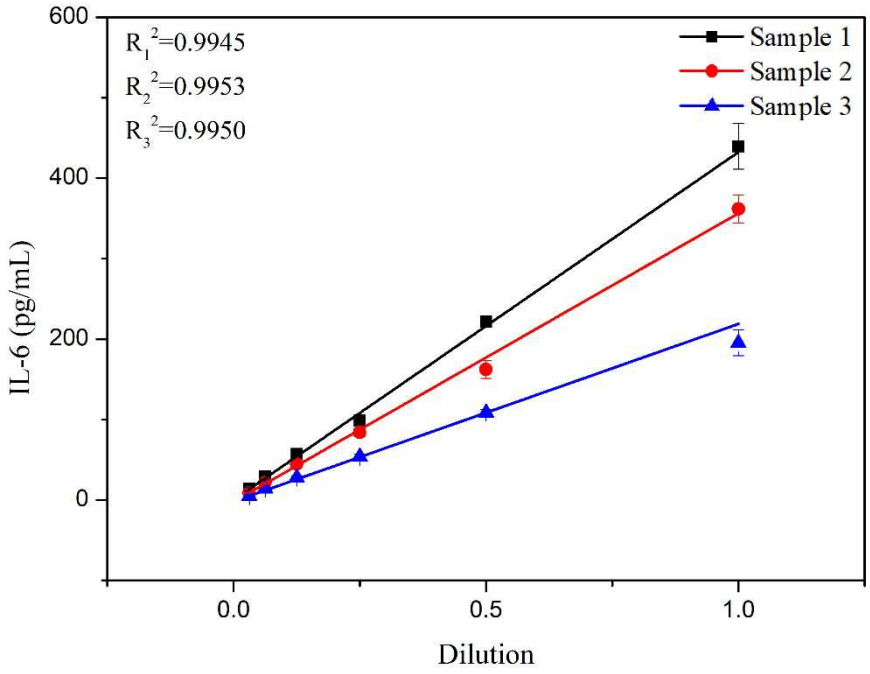

Fig. 3. Dilution linearity for IL-6 based on measurement of three positive serum samples

3.4 Analytical Sensitivity and Linearity

The standard curve for the assay was obtained based on the measurement of twelve serial concentrations of IL-6 standards (0, 2, 5,10, 20, 50,100,200, 500, 600, 700 and 1000 pg/mL), which were accurately prepared in sample dilution buffer, and each concentration took three replicates. After calculating the fluorescence intensities, we drew a broken line graph of the ratio between the area of the test and the control line (H_T/H_C) ratio about the concentration of IL-6 (Fig.4). We found that when the concentration of IL-6 was above 500 pg/mL, H_T/H_C ratio and the concentration of IL-6 were not linear. So the linear range of the LFIA strips ranges from 2 to 500 pg/mL.

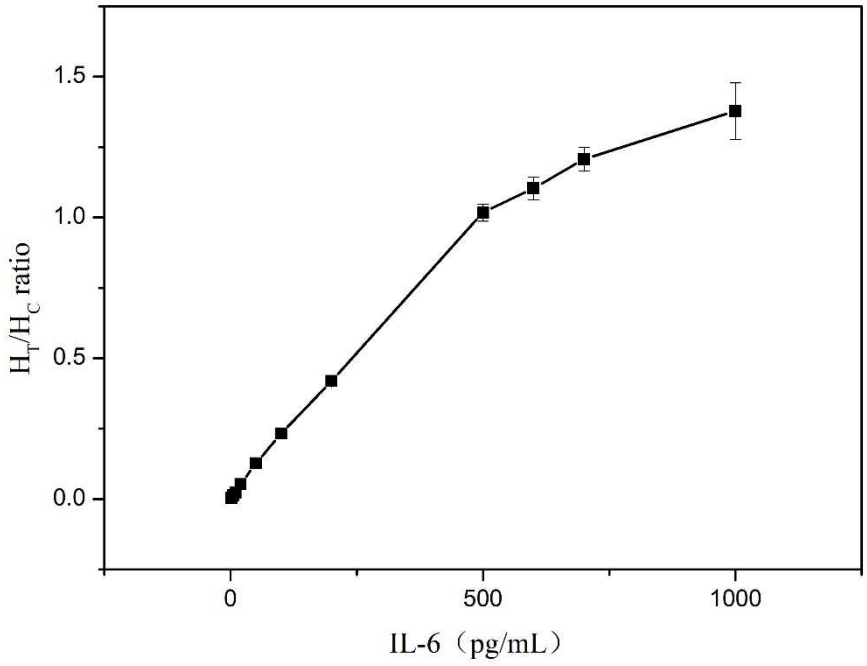

Fig.4. Determination of detection upper limit

We obtained a standard curve by plotting the H_T/H_C ratio against standard IL-6 concentrations as represented by the equation: y= 0.0020x+ 0.0051 (r = 0.9994) , where x (pg/mL) is the concentration of IL-6 and y is the H_T/H_C ratio, and the concentrations of IL-6 in samples could be quantitatively detected according to the equation (Fig.5). The analytical sensitivity was calculated by using the following: $LOD=2*3S_0/(X_2-X_0)$, where 2 was the numerical value of the 2 pg/mL standard, S_0 was the standard deviation (SD) of 0 pg/mL standard, X_2 was the average value of the H_T/H_C ratio of 2 pg/mL standard, X_0 is the average value of the H_T/H_C ratio of 0 pg/mL standard. The analytical sensitivity of the proposed assay was 0.37 pg/mL (n=10). These results showed the excellent performance of the developed LFIA test strips.

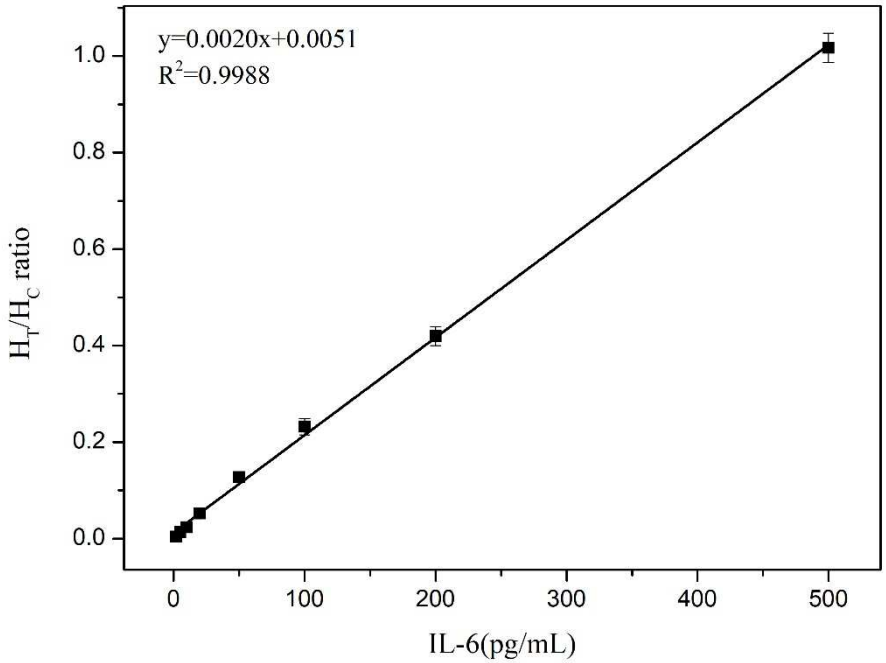

Fig. 5. Standard curve for IL-6

3.5 Accuracy

The accuracy of the assay was evaluated by a recovery test. A serum sample with a high concentration of IL-6 (380 pg/mL, measured by SIEMENS IL-6 kit) was diluted into three dilutions with IL-6-free human serum. The recovery ratios (measured value/expected value) were calculated. The results showed that they ranged from 1.02 and 1.06 (Table 2).

Table 2

Accuracy results of the IL-6 test strips (n = 3).

Sample	1:2	1:4	1:8
Expectation (ng/mL)	190	95	47.5
Mean value ± SD (ng/mL)	193.80±10.21	101.01±1.81	50.52±1.09
Recovery	1.02	1.06	1.06

3.6 Precision

The precision of the test strip was evaluated based on intra-day and inter-day assays using three reference standards (5, 20 and 50 pg/mL). Three reference standards were measured ten times in a day for the intra-assay study. As for the inter-assay study, three reference standards were measured in two consecutive days with ten replicates. The results are shown in Table 3. The intra- and inter-assay CVs were 5.92% and 7.68%

at 5 pg/mL, 6.29% and 7.59% at 20 pg/mL and 8.87% and 9.04% at 50 pg/mL, respectively. All the CVs were <10%, which demonstrated an acceptable level of reproducibility for IL-6 strip quantitation.

Table 3
Precision results of the IL-6 test strips.

Standards (pg/mL)	Intra-assay precision (n=10)		Inter-assay precision (n=10)	
	Mean ± SD (pg/mL)	CV%	Mean ± SD(pg/mL)	CV%
5	4.95±0.29	5.92	4.98±0.38	7.68
20	19.36±1.22	6.29	19.32±7.59	7.59
50	51.31±4.55	8.87	50.87±4.60	9.04

3.7 Specificity

To evaluate the specificity of the test strips for IL-6 determination, the CRP (53.4 mg/L), IL-8 (260 pg/mL) and procalcitonin (109.81 ng/mL) were measured three replicates respectively. All of the results were less than 2 pg/mL (Table 4). The results indicated that the developed IL-6 test strips were highly specific toward IL-6 and the interferents had insignificant effects on the results.

Table 4
Specificity results of the IL-6 test strips (n=3)

Interferent	Concentration of interferent	Test Results (pg/mL)
PCT	109.81 ng/mL	< 2
IL-8	6098 pg/mL	< 2
CRP	53.4 mg/L	< 2

3.8 Clinical Samples Analysis

In order to compare the two methods, a total of 214 patients' serum samples were measured with the developed LFIA strips and SIEMENS CLIA IL-6 kit. The results of correlation analysis were shown in Fig.6. A high correlation was obtained between the two assays ($y = 0.9502 x + 6.1397$, $r = 0.9757$, $p < 0.01$), where x represents the IL-6 concentrations measured by the SIEMENS CLIA IL-6 kit and y represents the values obtained by the developed Eu-np test strip. Therefore, the developed LFIA combined with Eu-np for IL-6 showed satisfactory performance compared to SIEMENS CLIA IL-6 kit.

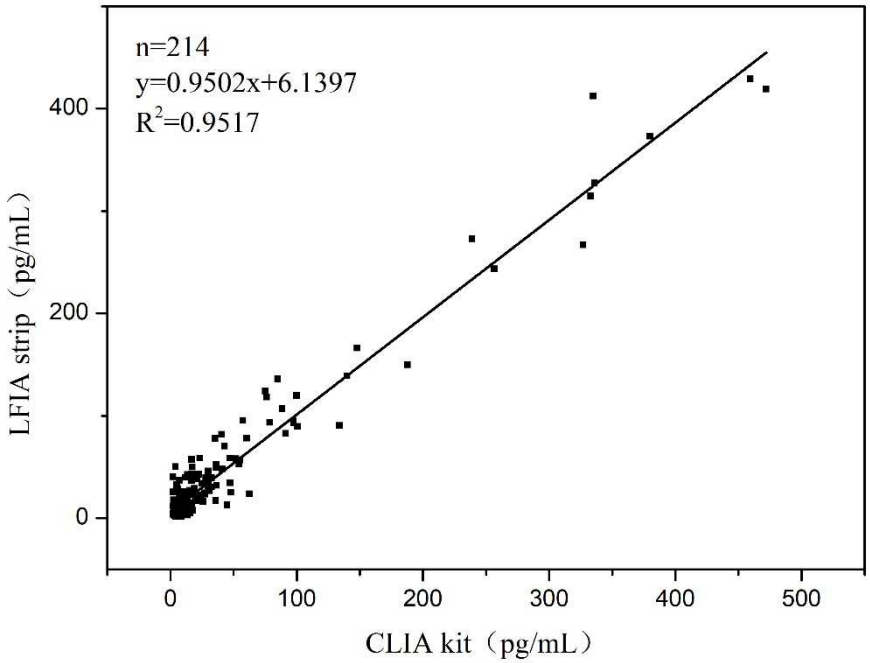

Fig. 6. Comparison of IL-6 in 214 sera measured with the proposed method and the CLIA kit.

4 Discussions

In this work, facile, fast, simple, sensitive, low-cost and reliable Eu-np-based LFIA has been developed for the quantitation of IL-6 in human serum. The europium nanoparticles were successfully applied to lateral flow tests strips, thus serving as a powerful fluorescence reporter. The LFIA strips are advantageous, not only for the relatively low cost, simplicity and rapidity of conventional lateral flow strips, but also for the high sensitivity, good specificity and photostability afforded by the europium nanoparticles.

There are several advantages of using europium nanoparticles as labels to detect IL-6. First, compared with the extensive and mature assay like ELISA and CLIA, the Eu-np-based LFIA strips only need 15 minutes to achieve the results. Second, with only two simple steps, adding serum samples and analyzing the result by a portable fluorescence strip reader, which is friendly to use. Third, the LFIA strips only need 70 μL of sample. Last, on count of the excellent features of the Eu-np, the strips get a good sensitivity (0.37 pg/mL), which is far below the concentration of IL-6 (about 6 pg/mL) in healthy individuals [43].

However, there's also a problem that needs to be solved. Patients in severe sepsis may have a high concentration above 500pg/mL [44], which is beyond the linear range. In future, more work remains to be done to address the hook effect.

5 Conclusions

In this study, we successfully developed a sandwich format LFIA for the detection of IL-6 in human serum based on Eu-np. The LFIA strips had a wide range (2-500 pg/mL) and a good sensitivity (0.37 pg/mL). The intra-assay CV and the inter-assay CV were 5.92%–8.87% and 7.59%–9.04%, respectively. The recovery rates ranged from 102% to 106%. Furthermore, a high correlation ($n = 214$, $r = 0.9756$, $p < 0.01$) was obtained when compared with the SIEMENS CLIA IL-6 kit. The LFIA strips only need 15 min to achieve the results with only 70 μL of sample. The LFIA strips are very suitable for the quantitative determination of IL-6, suggesting that the methodology should be applicable to the measurement of other biomarkers and for rapid quantitative detection in point-of-care test.

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References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G.R. Bernard, J.-D. Chiche, C.M. Coopersmith, R.S. Hotchkiss, M.M. Levy, J.C. Marshall, G.S. Martin, S.M. Opal, G.D. Rubenfeld, T. van der Poll, J.-L. Vincent, D.C. Angus, The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), JAMA 315 (2016) 801–810.

[2] R.S. Hotchkiss, L.L. Moldawer, S.M. Opal, K. Reinhart, I.R. Turnbull, J.-L. Vincent, Sepsis and septic shock, Nature reviews. Disease primers 2 (2016) 16045.

[3] D.W. Jekarl, S.-Y. Lee, J. Lee, Y.-J. Park, Y. Kim, J.H. Park, J.H. Wee, S.P. Choi, Procalcitonin as a diagnostic marker and IL-6 as a prognostic marker for sepsis, Diagnostic microbiology and infectious disease 75 (2013) 342–347.

[4] M.B. Mat-Nor, A. Md Ralib, N.Z. Abdulah, J.W. Pickering, The diagnostic ability of procalcitonin and interleukin-6 to differentiate infectious from noninfectious systemic inflammatory response syndrome and to predict mortality, Journal of critical care 33 (2016) 245–251.

[5] A. Kumar, D. Roberts, K.E. Wood, B. Light, J.E. Parrillo, S. Sharma, R. Suppes, D. Feinstein, S. Zanotti, L. Taiberg, D. Gurka, A. Kumar, M. Cheang, Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock, Critical care medicine 34 (2006)

1589–1596.
[6] J.D. Faix, Biomarkers of sepsis, Critical reviews in clinical laboratory sciences 50 (2013) 23–36.
[7] M. Naffaa, B.F. Makhoul, A. Tobia, M. Kaplan, D. Aronson, Z.S. Azzam, W. Saliba, Procalcitonin and interleukin 6 for predicting blood culture positivity in sepsis, The American journal of emergency medicine 32 (2014) 448–451.
[8] L. Weidhase, D. Wellhöfer, G. Schulze, T. Kaiser, T. Drogies, U. Wurst, S. Petros, Is Interleukin-6 a better predictor of successful antibiotic therapy than procalcitonin and C-reactive protein? A single center study in critically ill adults, BMC infectious diseases 19 (2019) 150.
[9] M. Feng, T. Sun, Y. Zhao, H. Zhang, Detection of Serum Interleukin-6/10/18 Levels in Sepsis and Its Clinical Significance, Journal of clinical laboratory analysis 30 (2016) 1037–1043.
[10] B. Sun, L.-F. Liang, J. Li, D. Yang, X.-B. Zhao, K.-G. Zhang, A meta-analysis of interleukin-6 as a valid and accurate index in diagnosing early neonatal sepsis, International wound journal 16 (2019) 527–533.
[11] V. Miguel-Bayarri, E.B. Casanoves-Laparra, L. Pallás-Beneyto, S. Sancho-Chinesta, L.F. Martín-Osorio, C. Tormo-Calandín, D. Bautista-Rentero, Prognostic value of the biomarkers procalcitonin, interleukin-6 and C-reactive protein in severe sepsis, Medicina Intensiva (English Edition) 36 (2012) 556–562.
[12] J.-J. Ríos-Toro, M. Márquez-Coello, J.-M. García-Álvarez, A. Martín-Aspas, R. Rivera-Fernández, A. Sáez de Benito, J.-A. Girón-González, Soluble membrane receptors, interleukin 6, procalcitonin and C reactive protein as prognostic markers in patients with severe sepsis and septic shock, PloS one 12 (2017) e0175254.
[13] X. Liu, A.J. Teichtahl, I.P. Wicks, Interleukin-6 In Rheumatoid Arthritis - From The Laboratory To The Bedside, Current Pharmaceutical Design 21 (2015) 2187–2197.
[14] X. Yao, J. Huang, H. Zhong, N. Shen, R. Faggioni, M. Fung, Y. Yao, Targeting interleukin-6 in inflammatory autoimmune diseases and cancers, Pharmacology & therapeutics 141 (2014) 125–139.
[15] T. Kishimoto, IL-6: from its discovery to clinical applications, International immunology 22 (2010) 347–352.
[16] M. Song, J.A. Kellum, Interleukin-6, Critical care medicine 33 (2005) S463-5.
[17] M. Helle, L. Boeije, E.d. Grrot, A.d. Vos, L. Arden, Sensitive ELISA for interleukin-6 Detection of IL-6 in biological fluids: synovial fluids and sera, Journal of immunological methods 138 (1991) 47–56.
[18] L. Luo, Z. Zhang, L. Hou, J. Wang, W. Tian, The study of a chemiluminescence immunoassay using the peroxyoxalate chemiluminescent reaction and its application, Talanta 72 (2007) 1293–1297.
[19] T. Li, M. Yang, Electrochemical sensor utilizing ferrocene loaded porous polyelectrolyte nanoparticles as label for the detection of protein biomarker IL-6, Sensors and Actuators B: Chemical 158 (2011) 361–365.
[20] D. Quesada-González, A. Merkoçi, Nanoparticle-based lateral flow biosensors, Biosensors & bioelectronics 73 (2015) 47–63.

[21] T. Salminen, A. Knuutila, A.-M. Barkoff, J. Mertsola, Q. He, A rapid lateral flow immunoassay for serological diagnosis of pertussis, Vaccine 36 (2018) 1429–1434.

[22] T.-H. Chou, C.-Y. Chuang, C.-M. Wu, Quantification of Interleukin-6 in cell culture medium using surface plasmon resonance biosensors, Cytokine 51 (2010) 107–111.

[23] Y. Wang, J. Sun, Y. Hou, C. Zhang, D. Li, H. Li, M. Yang, C. Fan, B. Sun, A SERS-based lateral flow assay biosensor for quantitative and ultrasensitive detection of interleukin-6 in unprocessed whole blood, Biosensors & bioelectronics 141 (2019) 111432.

[24] T. Yang, S. Wang, H. Jin, W. Bao, S. Huang, J. Wang, An electrochemical impedance sensor for the label-free ultrasensitive detection of interleukin-6 antigen, Sensors and Actuators B: Chemical 178 (2013) 310–315.

[25] H. Chen, T.K. Choo, J. Huang, Y. Wang, Y. Liu, M. Platt, A. Palaniappan, B. Liedberg, A.I.Y. Tok, Label-free electronic detection of interleukin-6 using horizontally aligned carbon nanotubes, Materials & Design 90 (2016) 852–857.

[26] J. Huang, J. Harvey, W.D. Fam, M.A. Nimmo, I.A. Tok, Novel Biosensor for InterLeukin-6 Detection, Procedia Engineering 60 (2013) 195–200.

[27] Y. Lou, T. He, F. Jiang, J.-J. Shi, J.-J. Zhu, A competitive electrochemical immunosensor for the detection of human interleukin-6 based on the electrically heated carbon electrode and silver nanoparticles functionalized labels, Talanta 122 (2014) 135–139.

[28] I. Ojeda, M. Moreno-Guzmán, A. González-Cortés, P. Yáñez-Sedeño, J.M. Pingarrón, Electrochemical magnetoimmunosensor for the ultrasensitive determination of interleukin-6 in saliva and urine using poly-HRP streptavidin conjugates as labels for signal amplification, Analytical and bioanalytical chemistry 406 (2014) 6363–6371.

[29] G.-C. Fan, X.-L. Ren, C. Zhu, J.-R. Zhang, J.-J. Zhu, A new signal amplification strategy of photoelectrochemical immunoassay for highly sensitive interleukin-6 detection based on TiO2/CdS/CdSe dual co-sensitized structure, Biosensors & bioelectronics 59 (2014) 45–53.

[30] M. Tertiş, B. Ciui, M. Suciu, R. Săndulescu, C. Cristea, Label-free electrochemical aptasensor based on gold and polypyrrole nanoparticles for interleukin 6 detection, Electrochimica Acta 258 (2017) 1208–1218.

[31] J. Peng, J. Guan, H. Yao, X. Jin, Magnetic colorimetric immunoassay for human interleukin-6 based on the oxidase activity of ceria spheres, Analytical biochemistry 492 (2016) 63–68.

[32] N. Liu, H. Yi, Y. Lin, H. Zheng, X. Zheng, D. Lin, H. Dai, Combined electrochemiluminescent and electrochemical immunoassay for interleukin 6 based on the use of TiO2 mesocrystal nanoarchitectures, Mikrochimica acta 185 (2018) 277.

[33] E.B. Bahadır, M.K. Sezgintürk, Lateral flow assays: Principles, designs and labels, TrAC Trends in Analytical Chemistry 82 (2016) 286–306.

[34] A. Chen, S. Yang, Replacing antibodies with aptamers in lateral flow

immunoassay, Biosensors & bioelectronics 71 (2015) 230–242.
[35] G.B. Aktas, J.H. Wichers, V. Skouridou, A. van Amerongen, L. Masip, Nucleic acid lateral flow assays using a conjugate of a DNA binding protein and carbon nanoparticles, Mikrochimica acta 186 (2019) 426.
[36] X. Mao, W. Wang, T.E. Du, Dry-reagent nucleic acid biosensor based on blue dye doped latex beads and lateral flow strip, Talanta 114 (2013) 248–253.
[37] C. Huang, Q. Wei, Q. Hu, T. Wen, L. Xue, S. Li, X. Zeng, F. Shi, Y. Jiao, L. Zhou, Rapid detection of severe fever with thrombocytopenia syndrome virus (SFTSV) total antibodies by up-converting phosphor technology-based lateral-flow assay, Luminescence : the journal of biological and chemical luminescence 34 (2019) 162–167.
[38] Y. Chen, Q. Fu, J. Xie, H. Wang, Y. Tang, Development of a high sensitivity quantum dot-based fluorescent quenching lateral flow assay for the detection of zearalenone, Analytical and bioanalytical chemistry 411 (2019) 2169–2175.
[39] T. Salminen, E. Juntunen, S.M. Talha, K. Pettersson, High-sensitivity lateral flow immunoassay with a fluorescent lanthanide nanoparticle label, Journal of immunological methods 465 (2019) 39–44.
[40] S.L. Znoyko, A.V. Orlov, A.V. Pushkarev, E.N. Mochalova, N.V. Guteneva, A.V. Lunin, M.P. Nikitin, P.I. Nikitin, Ultrasensitive quantitative detection of small molecules with rapid lateral-flow assay based on high-affinity bifunctional ligand and magnetic nanolabels, Analytica Chimica Acta 1034 (2018) 161–167.
[41] X. Huang, Z.P. Aguilar, H. Xu, W. Lai, Y. Xiong, Membrane-based lateral flow immunochromatographic strip with nanoparticles as reporters for detection: A review, Biosensors & bioelectronics 75 (2016) 166–180.
[42] I. de Souza Sene, V. Costa, G.E.N. Pauli, I.H. Bechtold, L.C.C. Nunes, C. Eiras, C.H.N. Costa, Lateral Flow Assay for Interleukin 6: A Technological and Scientific Prospection of a 10-Year Survey, Recent patents on biotechnology 12 (2018) 221–228.
[43] X. Chen, T. Dong, X. Wei, Z. Yang, N.M. Matos Pires, J. Ren, Z. Jiang, Electrochemical methods for detection of biomarkers of Chronic Obstructive Pulmonary Disease in serum and saliva, Biosensors & bioelectronics 142 (2019) 111453.
[44] C. Russell, A.C. Ward, V. Vezza, P. Hoskisson, D. Alcorn, D.P. Steenson, D.K. Corrigan, Development of a needle shaped microelectrode for electrochemical detection of the sepsis biomarker interleukin-6 (IL-6) in real time, Biosensors & bioelectronics 126 (2019) 806–814.

Highlights

- Simple and rapid lateral flow immunoassay based on europium nanoparticles.
- Quantitative detection of interleukin-6 in human serum.
- An analysis of clinical samples is used to get the reliable assay results.