Development of a Europium Nanoparticles Lateral Flow Immunoassay for NGAL Detection in Urine and Diagnosis of Acute Kidney Injury

Moli Yin
Jilin Medical University
Yuanwang Nie
Jilin Medical University
Hao Liu
Jilin Medical University
Lei Liu
Jilin Medical University
Lu Tang
Jilin Medical University
Yuan Dong
Jilin Medical University
Chuanmin Hu
Jilin Medical University
Huiyan Wang (jlmpcwhy@163.com)
Jilin Medical University

Research Article

Keywords: neutrophil gelatinase-associated lipocalin (NGAL), monoclonal antibody, lateral flow immunoassay, acute kidney injury (AKI)

Posted Date: March 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-331019/v1

Version of Record: A version of this preprint was published at BMC Nephrology on January 14th, 2022.
See the published version at https://doi.org/10.1186/s12882-021-02493-w.

Development of a europium nanoparticles lateral flow immunoassay for NGAL detection in urine and diagnosis of acute kidney injury

Moli Yin[1], Yuanwang Nie[2], Hao Liu[2], Lei Liu[1], Lu Tang[1], Yuan Dong[2], Chuanmin Hu[1], Huiyan Wang[1],*

[1]Jilin Collaborative Innovation Center for Antibody Engineering, Jilin Medical University, Jilin, Jilin 132013, PR China

[2]Academy of laboratory, Jilin Medical University, Jilin, Jilin 132013, PR China

* Author for correspondence: jlmpcwhy@163.com

Abstract

Background:AKI is related to severe adverse outcomes and mortality with Coronavirus Infection Disease 2019 (COVID-19) patients, that early diagnosed and intervened is imperative. Neutrophil gelatinase-associated lipocalin (NGAL) is one of the most promising biomarkers for detection of acute kidney injury (AKI), but current detection methods are inadequacy, so more rapid, convenient and accuracy methods are needed to detect NGAL for early diagnosis of AKI. Herein, we established a rapid, reliable and accuracy lateral flow immunoassay based on europium nanoparticles (Eu-NPS-LFIA) for the detection of NGAL in human urine specimens.

Methods:A double-antibody sandwich immunofluorescent assay using europium doped nanoparticles was employed and the NGAL monoclonal antibodies conjugate as labels were generated by optimizing electric fusion parameters. Eighty-three urine samples were used to evaluate the clinical application efficiency of this method.

Results: The quantitative detection range of NGAL in AKI was 1-3000 ng/mL, and the detection sensitization was 0.36 ng/mL. The CV of intra-assay and inter-assay were 2.57%-4.98% and 4.11%-7.83%, respectively. Meanwhile, the correlation coefficient between Eu-NPS-LFIA and ARCHITECT analyzer was significant (R^2=0.9829, n=83, p<0.01).

Conclusions: Thus, a faster and easier operation quantitative assay of NGAL for AKI has been established, which is very important and meaningful to diagnose the early AKI, suggesting that the assay can provide an early warning of final outcome of disease.

Keyworlds: neutrophil gelatinase-associated lipocalin (NGAL), monoclonal antibody, lateral flow immunoassay, acute kidney injury (AKI)

Background

Coronavirus Infection Disease 2019 (COVID-19) has widely spread in the worldwide scale with serious disaster[1].Acute kidney injury (AKI) has a higher rate of morbidity and mortality in common complication for critical illnesses and counted about 5%-7% of hospitalized patients in world[2].Several studies have evaluated the development of AKI is more strongly related to worse outcomes and mortality rates of COVID-19, described incidence of AKI that ranges widely from 0.5 to 36.6% in COVID-19 patients[3].Early detection and precise treatments of AKI can implement better preventive strategies and prevent deterioration of renal function and renal failure, effectively contain progression of the COVID-19 hospitalized patients[4]. Current diagnosis of AKI is confirmed by the concentration of serum creatinine (SCr), which is steady unless at least 50% of damaged kidney function[5]. Thus, several novel biomarkers were developed to improve the diagnostic specificity in early stages of AKI[6]. Among them, neutrophil gelatinase-associated lipocalin (NGAL) has been recognized as one of the promising biomarkers candidate for detection of AKI. NGAL is a 25 kDa glycoprotein associated with gelatinase from neutrophil and usually exist at lower level in human tissues such as stomach, colon and kidney, but its expression is dramatical increased in serum and urine when the kidney was with ischemic or nephrotoxic injury[7]. In AKI, several studies have discovered NGAL concentration in urine is significantly associated with SCr concentration[8]. In recent years, various electrochemical and immunological methods have been developed for the detection of NGAL, instance of electrochemical determination, solid-phase proximity ligation assay and enzyme-free electrochemical immunoassay[9, 10]. However, these techniques require precise equipment, specialized personnel and professional interpretation of the results. Therefore, to develop an effective and convenient detection assay for NGAL concentration in the urine is critical for this disorder monitoring.

Lateral flow immunoassays (LFIA) has been regard as desired screening assays on account of simplicity, in-situ analysis and easy to work[11]. The LFIA with fluorescent microparticles have already been used for detection of various microbial pathogens and several inflammation markers[12, 13]. Due to urine contain low viscosity and low content of solids so that it would be highly compatible with the LF platform, hence urine has been successfully examined in analysis of pregnancy tests, Schistosoma antigens and albumin[14]. The high sensitivity nanoparticles labeled in LFIA as fluorescent probes has been continually researched over past decades, gold nanoparticles are the most widely used, but the application of the lateral flow tests based on traditional labels is limitedfor there poor identification and weaker the signal[15]. Several novel nanoparticles have been generally applied to improve the sensitivity of LFIA, including carbon nanoparticles, quantum dots, fluorescent dyes, magnetic nanolabels and europium (III) dye-doped nanoparticles[16]. The fluorescent EU-NPS (III) as carriers can improve 100-fold sensitivity contrast with colloidal gold nanoparticles labeled in LFIA[17]. EU-NPS with long fluorescence lifetimes, strong stability, high precision and not disturb the sample quality, have used in sandwich-type immunoassays of medical diagnostics recently[18]. Currently there are antibodies available for the development of NGAL have been applied to different platforms for NGAL detection, but not available in LFIA based on EU-NPS labels experimented on the detection of NGAL in addition to UCP technology-based lateral flow assay [19, 20].

Here, we established a new method with EU-NPS as labels of LFIA for the rapid, sensitive and early measurement of NGAL in urine based on two mAbs 1G1 and 2F4 which are discoveried by our lab.The method is double-antibody sandwich immunofluorescent assay using europium doped nanoparticles and NGAL monoclonal antibody conjugate as labels. The mAb 1G1 was conjugated with fluorescent nanoparticles and the mAb 2F4 was used to capture EU-NPS-1G1-antigen complex in T-line. Our results showed that EU-NPS-LFIA could be used for the early NGAL detection in urine and allow improvement in the treatment of SARS-CoV-2 patients.

Methods

Urine samples

The total of 83 Human urine samples were harvested from Affiliated Hospital of Jilin Medical University. These samples were stored at -80°C. The ethical guidelines were strictly complied in the experiment, was provided by the Affiliated Hospital of Jilin Medical University. All subjects were received informed consent for the study of urine samples.

Expression and purification of NGAL

The human NGAL gene sequence from Genbank (NP_005555.2) was synthesized by BGI Genomics and added restriction enzymes *Hind*III and *Xho*I at both ends. The plasmids were digested with *Hind*III and *Xho*I, and then cloned into the pSecTag2A vector. The constructed plasmids were sequenced to confirm without mutation, and then transformed into CHO cells by the Lipofectamine^{TM}2000. After 8 days, the cell supernatant was collected after filtering 0.45μm filter. The expressed NGAL-6×His protein was purified with the Ni-NTA column, and the different fractions were collected and appraised by sodium dodecyl sulfate-polyacrylamide gel electrophoresis （SDS-PAGE）. The BCA Protein Quantification Kit measured the concentration of protein.

The purified recombinant protein was subjected in 12% SDS-PAGE, then adsorbed onto a polyvinylidene fluoride (PVDF) membrane. After the membrane was blocked in tris buffer with 1% Tween-20 (TBST) solution containing 5% skimmed milk at RT for 2 h, incubated with HRP-conjugated anti-6×His tag antibody (1:5000) in the dark for 1 h at RT, visualized with Benzidine after washed with TBST and visualized by Bio-Rad Western blotting detection system (DNR Bio-Imaging Systems Ltd., Israel).

Generation and purification of Monoclonal antibodies

In the first immunization, the female BALB/c mice (age of 6 weeks, a total of 10) were immunized with 50μg NGAL-6×His recombinant protein emulsified in equal

dosage of complete Freund's adjuvant, and accessional immunized were accomplished with protein emulsified in incomplete Freund's adjuvant. Two hypodermic injections on the back of mice and subsequent were intraperitoneal injections spaced 21 days. The serum samples were collected one week after the third injection, and the titre of antiserum was determined with indirect enzyme-linked immunosorbent assay (ELISA)[21]. Three days before fusion, mice were performed to booster immunization with 50μg of NGAL-6×His diluted with 0.9% NaCl.

The isolated immune mice spleen cells and the SP2/0 myeloma cells mixed at a ratio of 3:1 to fuse in a platinum electrode LF498-3 fusion chamber (BEX Co., Ltd, Japan) as described literature[22]. Briefly, the mixed cell was washed twice with 10 mL electrofusion buffer (0.3 M mannitol, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, pH 7.2), re-suspended at a concentration of 2×10^7 cells/mL. The fusion was completed using an alternating current voltage of 50 V at 0.8 MHz for 20 s, direct current pulse voltage of 450 V of 2 repetitions for 0.5 s, and post-fusion was 50 V at 0.8 MHz for 7 s. Finally, the electric-treated cell suspension was moved from the fusion chamber into 4.5 mL of preheated RPMI 1640 (20% fetal bovine serum) for 30 min at 37℃, then cultured in 96-well plates and incubated with 5% CO_2 at 37℃. After 24 h, hypoxanthine-aminopterin-thymidine (HAT) was supplemented to each well. The Cell culture supernatants were screened by ELISA after 9 days fusion, and calculated number of hybridoma clones. The BALB/c mice which injected with paraffin oil in advance were inoculated with 1×10^6 of NGAL hybridoma cells, the ascites were purified by Protein A column.

Identification of monoclonal antibodies

The immunoglobulin subclasses of antibodies were analyzed using the antibody subclass identification kit. The indirect ELISA screened the specific monoclonal antibodies (MAbs) by using purified recombinant NGAL-6×His protein and PCT-6×His protein. The interaction between antigen and antibodies were determined with BIAcore T200 system (GE Healthcare, Stockholm, Sweden) in HBS-EP buffer (0.005% surfactant P20, 10 mM Hepes, pH 7.4, 3 mM EDTA, 150 mM NaCl). The

NGAL antigen was adsorbed on CM5 biosensor chips reaching 400-480 response units (RU) by an amine coupling kit. The antibodies (2F4 and 1G1) were diluted in HBS-EP buffer were slowly passed over the chip with 50 μL/min for 5 min, respectively, and subsequently HBS-EP buffer injected over the chip to monitor the dissociation phase for 4 min. The sensor chips were regenerated with Glycine solution (pH 3.0) following the dissociation phase. For each analyte passed over the chip, the specific responses from the antigen flow channel could subtract non-specific responses for the control flow channel. The fitted saturation binding curves were plotted based on concentrations of analyte for equilibrium binding responses to calculate KD.

The specificity of anti-NGAL monoclonal antibodies was determined by Western Blot. Briefly, the NGAL proteins were done to 12% SDS-PAGE, then adsorbed onto polyvinylidene fluoride (PVDF) membrane that activated by soaking in methanol for 15 s, and then subjected to the electrophoresis conditions in 100 V for 2 h. After blocking in Tris-HCl buffer with 1% Tween-20 solution (TBST) containing 5% skimmed milk at RT for 2 h, the membrane was incubated with mouse anti-NGAL mAb as the primary antibodies at 4°C. The next day, membrane was washed with TBST and incubated with anti-mouse conjugated HRP IgG in the dark for 1.5 hour at RT. Finally, the membrane was visualized by the Western blot detection system of enhanced chemiluminescence (ECL).

Conjugation of Europium nanoparticles

The Anti-NGAL monoclonal antibody 1G1 were covalently conjugated to EU-NPS with standard procedure of Bangs Laboratories. Briefly, 100 μL EU-NPS were added to 900 μL 0.05 M MES (pH 7.0) and dispersed by ultrasound, vibrated for 15 min at RT in the presence of 0.08 M N-hydroxysulfosuccinimide (NHS) and 0.05 M carbodiimide (EDC).The activated EU-NPS ware washed with coupling buffer (0.05 mM H_3BO_3, 0.04 mM $Na_2B_4O_7$, pH 7.5) and reacted with 0.3 mg antibody for 2.5 h at RT. The europium-conjugated compound was incubated in 1000 μL blocking buffer (10% BSA, 20% tween-20, 0.05 M Tris-HCl) for 1h, added to 1000 μL stock

solutions (10% BSA, 20% trehalose, 20% tween-20, 0.05 M Tris-HCl) to store at 4℃.

The development of LFIA

The Lateral flow immunochromatography strips were consisted of nitrocellulose membrane, conjugate pad, sample pad and absorbing pad. The glass fiber membranes were soaked in the blocking buffer (20% trehalose, 10% BSA, 20% tween-20, 0.05 M Tris, 3.2 mM EDTA.Na_2, pH 8.6) for 1.5 h. The concentration of 1 mg/mL mAb 2F4 was coated on the test line (TL) of nitrocellulose membrane, and goat-anti-mouse IgG 1 mg/mL was coated on the same NC membrane at a distance of 4 mm to form a quality control line (CL), the spray volume of dispenser instrument was set at 1 μL/cm. The membrane and glass fiber mat were dried for 48 h at 45℃ before tested. The 2 μL EU-NPS-1G1 conjugate particles were coated on the conjugate pad, and fluorescence signal was measured by immunofluorescent analyzer (Guangzhou Labsim Biotech Co., Ltd).

Clinical sample testing and analysis

The serial concentrations of NGAL standards antigen (10, 50, 100, 200, 400, 800, 1500, 2000 and 3000 ng/mL) were prepared by using FBS to strengthen specific reaction of bioconjugate, each concentration done three replicates. After the clinical samples were added onto the sample pads, the results of fluorescence intensity on the T line (HT) and the C line (HC) were recorded by the reader. Quantitative detection was completed by the HT/HC ratio to effectively eliminate strips (T and C) difference and matrsample standard matrix effects[23]. The standard curve was plotted against each concentration of NGAL and HT/HC ratio.

Statistical analysis

The Passing-Bablok regression analysis and Bland-Altman plot were performed by analysis of variance (ANOVA) of MedCalc and SPSS 17.0 software. All data were showed as mean value with standard deviation (mean ± S.D.).

Results

Expression and purification of recombinant NGAL protein

The pSecTag2A-NGAL recombinant plasmid was transfected into CHO cells. The NGAL-6×His protein mainly expressed in cell supernatant and subsequently purified by Ni Sepharose. The purified recombinant NGAL protein was obtained about 95% purity and analyzed by SDS-PAGE with molecular approximate weight of 23.7 kDa . Western blot also confirmed the recombinant protein NGAL expression and purification (Figure 1).

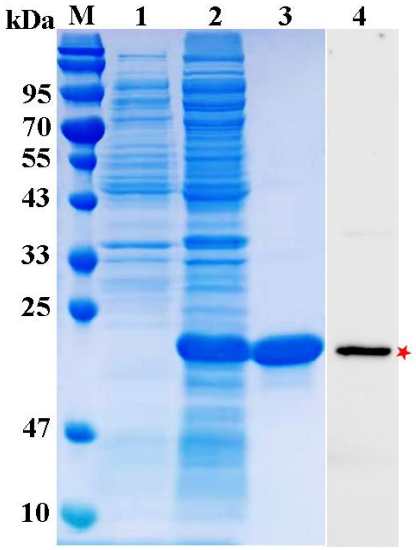


Figure 1. Expression and purification of NGAL-6×His. (A) Purification of NGAL-6×His protein were appraised by SDS-PAGE and Western blot, Lane M, protein marker; Lane 1, supernatant of CHO cell culture; Lane 2, supernatant of induced sample; Lane 3, purified protein ; Lane 4, Western blot analysis of NGAL-6×His expression.

Generation of monoclonal antibodies

First, we compared the effects of DC voltage on cell membrane perforation under different electric field intensities, and the pulse amplitude was 400 V, 450 V, 500 V and 550 V respectively, so as to optimize the electric fusion scheme. According to previous reports, cell concentration has a significant impact on fusion efficiency[24].

The mixed suspension of isolated spleen cells and SP2/0 myeloma cells was transferred into the fusion chamber at different concentrations of approximately 2×10^6, 2×10^7, and 2×10^8 cells /mL. The fusion efficiency was the highest at 450 V DC, and the optimal cell concentration was 2×10^7 cells/mL (Figure 2).

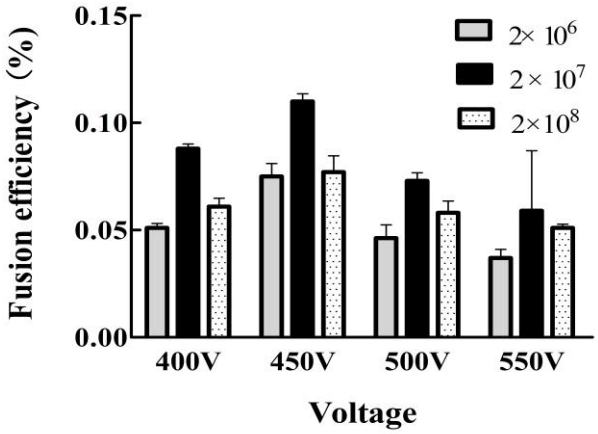


Figure 2. Comparison of fusion efficiency using different the puncture pulse height. The fusion cells were seeded into 96-well plates at approximately 2×10^5 cells/well. A total of 480 wells was assessed for each condition. The calculated method of the fusion efficiency (%): the total number of colonies in 480 wells were counted,then divided by the number of input B cells and multiplied by 100. The columns represent the average fusion efficiency (%) of 3 experiments, the error bars represent the SD.

Characterization of monoclonal antibodies

The mAbs were purified by Protein A column. The purified mAbs were separated by 12% SDS-PAGE, two bands with molecular weight 55 kDa and 25 kDa were observed. Western blot indicated that all monoclonal antibodies specifically bind to human NGAL protein (Figure 3A). After cell electrofusion and sub-cloning, the supernatants of hybridomas were detected by indirect ELISA with NGAL-6×His and PCT-6×His to ensure antibodies specific binding to the NGAL. Two highly positive hybridomas (1G1, 2F4) with specific NGAL binding while without cross-reaction to PCT-6×His were successfully selected for production and purification of mAbs (Figure 3B). To detect the affinity of two monoclonal antibodies, the interaction between antibodies with different concentrations and NGAL protein was analyzed by BIAcore T200 system. The kinetic diagram showed that the affinity of 2F4 and 1G1

were $4.5x10^{-7}$ and $6.0x10^{-7}$, respectively (Figure 3C and 3D). The isotypes of those two mAbs which were detected by commercial kits were IgG1.

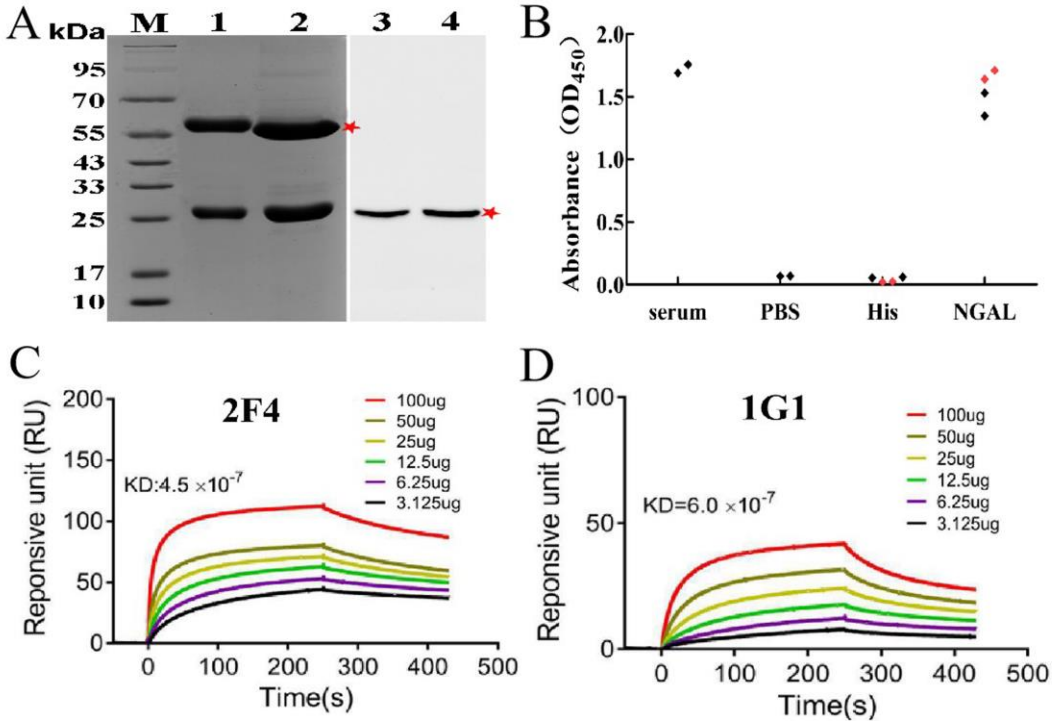


Figure 3. Characterization of monoclonal antibodies. (A) The ascites were assessed by SDS-PAGE and Western blot, Lane M, protein marker; Lane 1, purified the ascites of 2F4; Lane 2, purified the ascites of 1G1; Lane 3, Western blot analysis of mAb 2F4 ; Lane 4, Western blot analysis of mAb 1G1. **(B)** The Cross-reactivity of 2 hybridoma lines was tested by Indirect ELISA, NGAL-6×His and PCT-6×His were coated onto microtiter plates. The positive control was a NGAL-6×His immune serum; PBS were used as blank control. **(C)** Relative affinity of MAb 2F4. **(D)** Relative affinity of MAb 1G1.

EU-NPS-LFIA Procedures

Based on EU-NPS as labels and the sandwich-type immunoassay, the lateral flow immunoassay was established for detection of NGAL. As schematically illustrated (Figure 4), TL and CL of nitrocellulose membrane were coated with the monoclonal antibodies 2F4 and goat anti-mouse IgG. EU-NPS-1G1 was labeled on the conjugate pad. The sample containing NGAL antigen migrated towards the conjugate pad to combine with EU-NPS-1G1 and form antigen-antibody complexes. Subsequently, the complexes were captured by mAb 2F4 in T-line while migrating to form sandwich complexes. Excess complex was combined the goat anti-mouse IgG. Test strips were

measured with a fluorescence detector after 15 min.

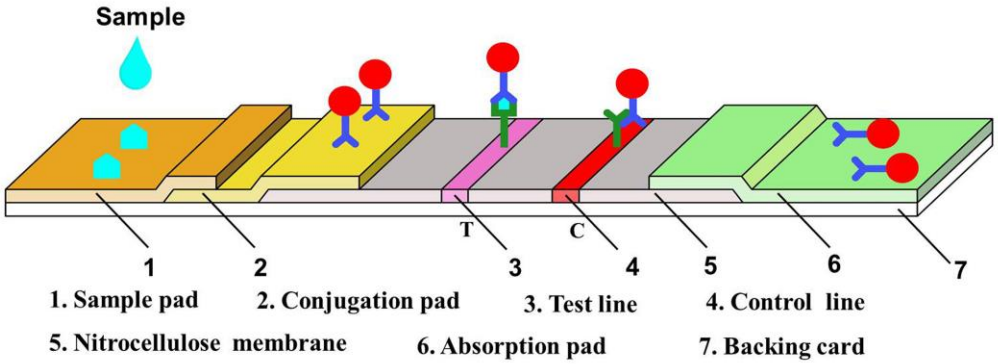


Figure 4. Schematic description of fluorescence immunoassay system employed Europium-conjugated NGAL mAbs.

Performance evaluation

The antibody pairs (2F4- labled1G1) as detector and capture antibodies were used to establish fluorescent immunochromatographic, and the analytical performances of EU-NPS-LFIA was evaluated by building the standard curve. The relative fluorescence intensity ratio(HT/HC) was increased with NGAL concentration. EU-NPS-1G1 showed reaction to mAb 2F4 (Figure 5A). The high-dose hook influence wasn't detected when concentration of antigen reached 3000 ng/mL. The regression equation was exhibited as follows: $y = 0.0012 x + 0.0059$ (R^2=0.99), where y represents the ratio of HT/HC , x represents the concentrations of NGAL (Figure 5B).The detection limit (LOD) was 0.36 ng/mL (3 times the standard deviation of the blank, n = 20) calculated by the Clinical Laboratory Standards Institute (CLSI) Guideline EP17-A2, the NGAL concentration had linear relationship in the range of 1-1500 ng/mL[25].

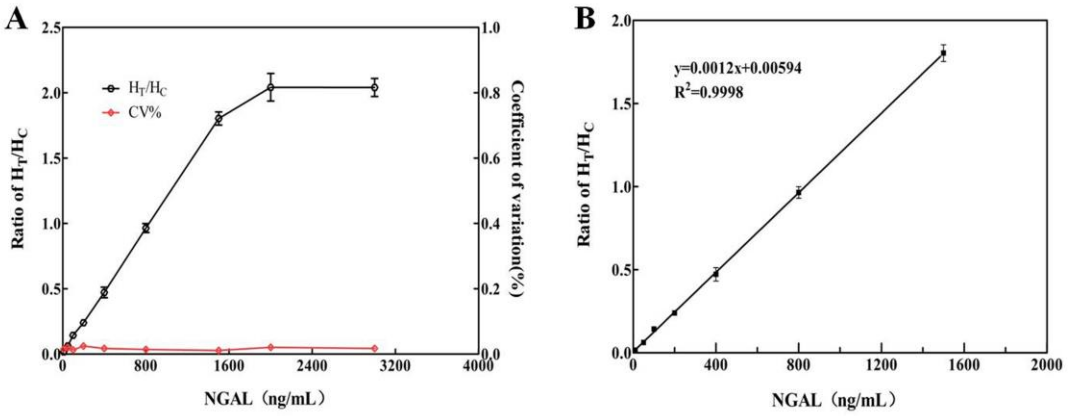


Figure 5. Standard curves for NGAL. (A) The ratio of HT/HC, by antibody pairing in LFIA, the antibody pairs 2F4-labled1G1 ratio of HT/HC rose with increasing concentration of NGAL. (B) The standard curve of NGAL.

Precision

Recovery experiments of the intra-assay and inter-assay were performed to evaluate precision and reproducibility of immunological method. The different concentrations (50, 200 and 800 ng/mL) of NGAL standard substance were added to negative urine samples. The intra-assay precision was calculated with three replicates at each spiked concentration in 1 day, and inter-assay precision was calculated with three replicates at each spiked concentration at every three days for fifteen days continuously[26]. The results were shown in **Table 1**, the calculated intra-assay CV ranged from 2.57% to 4.98% (n=10), lower than 10%. The inter-assay CV ranged from 4.11% to 7.83% (n=15), lower than 10% too. These results explained that the precision of the developed LFIA was a high level, and reproducibility was an acceptable level.

Table 1. Reproducibility analysis of the EU-NPS-LFIA test strip by intra-assay and inter-assay precision

NGAL (ng/mL)	Intra-Assay Precision (n = 10)		Inter-Assay Precision (n = 15)	
	Mean±SD (ng/mL)	CV (%)	Mean±SD (ng/mL)	CV (%)
50	49.57±0.003	4.98	51.28±0.037	5.68
200	199.18±0.009	3.84	202.78±0.019	7.83
800	799.76±0.025	2.57	801.68±0.039	4.11

Specificity

The specificity of the test strips was evaluated by adding endogenous substances in normal and different concentrations urine samples, including creatinine, glucose and urea nitrogen. As shown in **Table 2**, the results indicated that all relative deviations (RD) was in the range of ± 10% , suggesting antigen-antibody interaction were stable and illustrating the specificity of test strips was acceptable toward NGAL.

Table 2. The specificity study of the EU-NPS-LFIA with different interfering endogenous substances

Interfering Substance	NGAL (27.64ng/mL)		NGAL (53.72ng/mL)	
	Value	RD (%)	Value	RD (%)
control	0.037±0.002	0.54	0.069±0.003	2.35
Creatinine(10 mg/mL)	0.035±0.008	-4.55	0.067±0.009	-2.06
glucose(10 mg/mL)	0.036±0.007	-2.55	0.071±0.006	3.83
urea nitrogen(100 mg/mL)	0.036±0.004	2.72	0.064±0.008	-5.98

Note: RD = (Value-Standard value) / Standard value.

Clinical samples tests

In order to appraise applicative competence of the NGAL based on EU-NPS-LFIA for determination of clinical samples, a total of 83 urine samples containing 26 low value samples (11-70 ng/mL), 19 median value samples (110-800 ng/mL), 38 high value samples (800-1740 ng/mL), were measured on the ARCHITECT urine NGAL assay. The results of correlation coefficient (R^2) of the regression curve was 0.9829 (p<0.01), indicating that the two detection methods had a significant linear relationship (where x represents concentrations of NGAL obtained by the ARCHITECT analyzer, y represents values measured by developed test strips) (Figure 6). Thus, the developed EU-NPS-LFIA for determination of NGAL was very accurate in clinical testing.

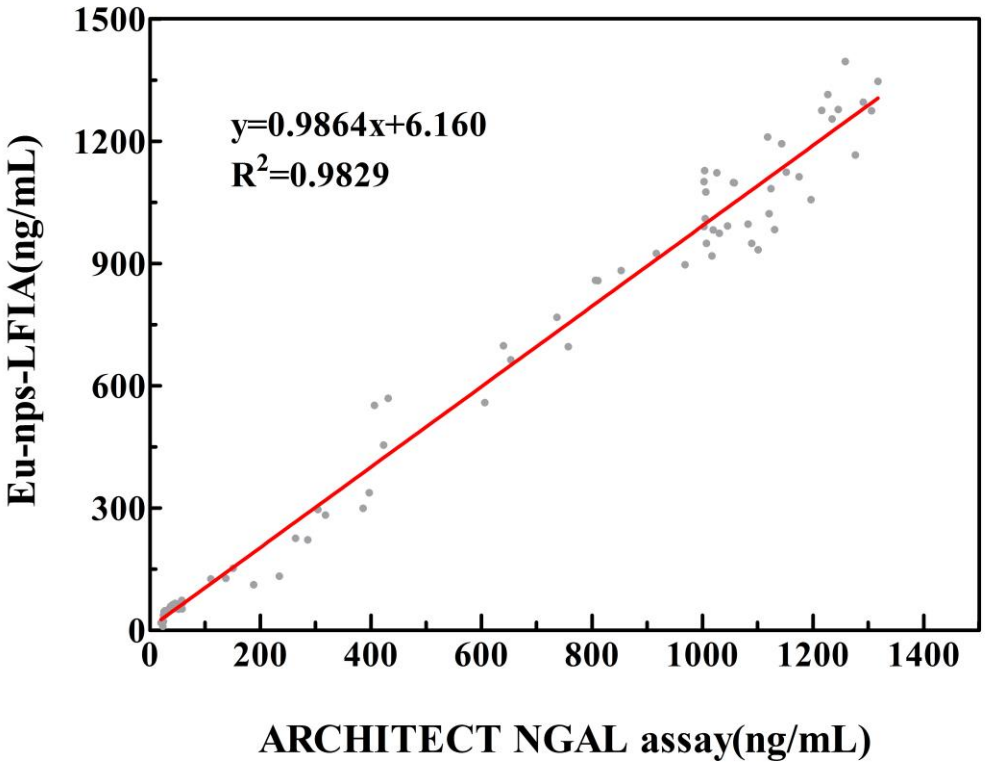


Figure 6. Comparison of EU-NPS-LFIA with ARCHITECT urine assay estimated correlation of the results for NGAL clinical test.

Discussion

The sensitivity europium-based lateral fluorescence immunoassays (EU-NPS-LFIA) for detection of NGAL in human urine and diagnosis of acute kidney injury during the COVID-19 pandemic has been developed in our study. AKI developed in 89% of severe patients with COVID-19, and a majority of patients had predominantly oliguria when appears earlier than plasma Creatinine[27]. Biomarkers increased elevated levels at admission, which associated with increased mortality. NGAL is one of these biomarkers, levels of which may positive correlation with risk for mortality in COVID-19 hospitalized patients[28]. The low efficiency of PEG fusion in conventional methods to causes difficulties in obtaining functional antibodies, we optimized electric fusion parameters that enabled enhancement of fusion efficiency to prepare of viable hybridomas, and obtained two anti-NGAL mAbs, one conjugated with EU-NPS while the other used as capture antibodies[24].

In recent years, many studies have been reported to develop several NGAL rapid diagnostic immunoassays, including the photoelectrochemical immunosensor and three electrochemical immunosensors, the solid-phase proximity ligation assay and lateral flow assay[29]. In one study, immunosensor has been made by NGAL capture antibodies immobilized to screen-printed-modified carbon electrode and labeled addition of secondary antibody against NGA to PB-NP-decorated g-C_3N_4 nanosheets forming a sandwich on the SPCE[10]. The other study developed antibody against NGAL was immobilized on a screen printed electrode (SPCE) modified with electropolymerized aniline deposited on top of an electrosprayed graphene/poly-aniline (G/PANI)[29]. Two reports showed the LOD varied widely from 0.6 pg/mL to 21.1 ng/mL, depending on the g-C_3N_4 nanosheets with N element enhanced electrocatalytic efficiency of the nanohybrids than graphene nanosheets, and the LOD of the NGAL assay varies depending on immunosensor, conjugated-complex and antibodies[30]. According to those studies, we enlightened that sensitivity of LFIA could be improved by basing on different fluorescence nanoparticles mentioned in the preamble section.

Fluorescence immunoassays have advantange in various detection fields, especially in mature quantum dots, that sensitivity was influenced by the monoclonal antibodies and fluorescent materials, and could greatly improve by EU-NPS and specific monoclonal antibodies to use the fluorescent probes[31]. The europium nanoparticles were applied to fluorescence lateral flow immunoassay strips had matured in the detection field, and enhanced several advantages of LFIA for assay sensitivity, specificity and stability[32]. Due to excellent properties of the EU-NPS that the limit of detection for NGAL in this method was 0.36 ng/mL, while the mean urine NGAL levels was about 7.0 ng/mL in healthy individuals[33]. The developments and application about the EU-NPS-LFIA of NGAL are still in initial phase, this assay need to test NGAL of serum samples with COVID- 19 patients for which improvement in the treatment in the future of works.

Conclusions

In the study, we successfully got two mouse anti-NGAL mAbs (2F4, 1G1) and were labeled with EU-NPS to establish the lateral flow immune technique. The EU-NPS-LFIA was found to detect NGAL in a wide range of 1-3000 ng/mL within 15 min, the detection sensitivity reached 0.36 ng/mL. These anti-NGAL mAbs could be reliability utilized in fluorescence lateral flow immunoassay of NGAL detection in the urine samples, so that it should be applicable in the AKI diagnosis.

Abbreviations

COVID-19: Coronavirus Infection Disease 2019; AKI: Acute kidney injury; SCr: serum creatinine; NGAL: neutrophil gelatinase-associated lipocalin; LFIA: Lateral flow immunoassays; EU-NPS (III): europium (III) dye-doped nanoparticles; ELISA: enzyme-linked immunosorbent assay; MAbs: monoclonal antibodies

Acknowledgements

Not applicable.

Authors' contributions

HYW designed the experiments; MLY, YWN, HL and LL performed study planning, data analysis, manuscript drafting and manuscript review.LT, YD and CMH revised the manuscript.All authors gave intellectual input to the manuscript and approved final version.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article:This work was supported by the 'Research and Development of Industrial Technology' Program of Jilin Province, PR China (grant nos. 20180623045TC and 20170204005YY), National Natural Science Foundation of China (no. 21827812) and National Training Program of Innovation and Entrepreneurship for Undergraduates (nos. 201913706017 and 202013706002).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

The study was performed on the basis of institutional ethical guidelines, approved by the Affiliated

hospital of Jilin Medical University in China. All procedures were followed the principles Declaration of Helsinki, informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

Author details

[1]Jilin Collaborative Innovation Center for Antibody Engineering, Jilin Medical University, Jilin, Jilin 132013, PR China. [2]Academy of laboratory, Jilin Medical University, Jilin, Jilin 132013, PR China.

References

1. Zheng X, Zhao Y, Yang L: **Acute Kidney Injury in COVID-19: The Chinese Experience**. *Seminars in nephrology* 2020, **40**(5):430-442.
2. Mandelbaum T, Scott D, Lee J, Mark R, Malhotra A, Waikar S, Howell M, Talmor D: **Outcome of critically ill patients with acute kidney injury using the Acute Kidney Injury Network criteria**. *Critical care medicine* 2011, **39**(12):2659-2664.
3. Al-Hwiesh A, Mohammed A, Elnokeety M, Al-Hwiesh A, Al-Audah N, Esam S, Abdul-Rahman I: **Successfully treating three patients with acute kidney injury secondary to COVID-19 by peritoneal dialysis: Case report and literature review**. *Peritoneal dialysis international* 2020, **40**(5):496-498.
4. Shabaka A, Rovirosa-Bigot S, Guerrero Márquez C, Alonso Riaño M, Fernández-Juárez G: **Acute kidney injury and nephrotic syndrome secondary to COVID-19-associated focal segmental glomerulosclerosis**. *Nefrologia* 2021.
5. Shapiro N, Trzeciak S, Hollander J, Birkhahn R, Otero R, Osborn T, Moretti E, Nguyen H, Gunnerson K, Milzman D *et al*: **The diagnostic accuracy of plasma neutrophil gelatinase-associated lipocalin in the prediction of acute kidney injury in emergency department patients with suspected sepsis**. *Annals of emergency medicine* 2010, **56**(1):52-59.e51.
6. Alge J, Arthur J: **Biomarkers of AKI: a review of mechanistic relevance and potential therapeutic implications**. *Clinical journal of the American Society of Nephrology* 2015, **10**(1):147-155.
7. Gabbard W, Milbrandt E, Kellum J: **NGAL: an emerging tool for predicting severity of AKI is easily detected by a clinical assay**. *Critical care* 2010, **14**(4):318.
8. Wagener G, Jan M, Kim M, Mori K, Barasch J, Sladen R, Lee H: **Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery**. *Anesthesiology* 2006, **105**(3):485-491.
9. Kannan P, Tiong H, Kim D: **Highly sensitive electrochemical determination of neutrophil gelatinase-associated lipocalin for acute kidney injury**. *Biosensors bioelectronics* 2012, **31**(1):32-36.

10. Zhang F, Zhong H, Lin Y, Chen M, Wang Q, Lin Y, Huang: **A nanohybrid composed of Prussian Blue and graphitic CN nanosheets as the signal-generating tag in an enzyme-free electrochemical immunoassay for the neutrophil gelatinase-associated lipocalin**. *Mikrochimica acta* 2018, **185**(7):327.
11. Zuk R, Ginsberg V, Houts T, Rabbie J, Merrick H, Ullman E, Fischer M, Sizto C, Stiso S, Litman D: **Enzyme immunochromatography--a quantitative immunoassay requiring no instrumentation**. *Clinical chemistry* 1985, **31**(7):1144-1150.
12. Juntunen E, Myyryläinen T, Salminen T, Soukka T, Pettersson K: **Performance of fluorescent europium(III) nanoparticles and colloidal gold reporters in lateral flow bioaffinity assay**. *Analytical biochemistry* 2012, **428**(1):31-38.
13. Swanson C, D'Andrea A: **Lateral flow assay with near-infrared dye for multiplex detection**. *Clinical chemistry* 2013, **59**(4):641-648.
14. Juntunen E, Arppe R, Kalliomäki L, Salminen T, Talha S, Myyryläinen T, Soukka T, Pettersson K: **Effects of blood sample anticoagulants on lateral flow assays using luminescent photon-upconverting and Eu(III) nanoparticle reporters**. *Analytical biochemistry* 2016, **492**:13-20.
15. Chen A, Yang S: **Replacing antibodies with aptamers in lateral flow immunoassay**. *Biosensors bioelectronics* 2015, **71**:230-242.
16. Chen Y, Fu Q, Xie J, Wang H, Tang Y: **Development of a high sensitivity quantum dot-based fluorescent quenching lateral flow assay for the detection of zearalenone**. *Analytical bioanalytical chemistry* 2019, **411**(10):2169-2175.
17. Zhang F, Zou M, Chen Y, Li J, Wang Y, Qi X, Xue Q: **Lanthanide-labeled immunochromatographic strips for the rapid detection of Pantoea stewartii subsp. stewartii**. *Biosensors bioelectronics* 2014, **51**:29-35.
18. Nankoberanyi S, Mbogo G, LeClair N, Conrad M, Tumwebaze P, Tukwasibwe S, Kamya M, Tappero J, Nsobya S, Rosenthal P: **Validation of the ligase detection reaction fluorescent microsphere assay for the detection of Plasmodium falciparum resistance mediating polymorphisms in Uganda**. *Malaria journal* 2014, **13**:95.
19. Li H, Mu Y, Yan J, Cui D, Ou W, Wan Y, Liu S: **Label-free photoelectrochemical immunosensor for neutrophil gelatinase-associated lipocalin based on the use of nanobodies**. *Analytical chemistry* 2015, **87**(3):2007-2015.
20. Lei L, Zhu J, Xia G, Feng H, Zhang H, Han Y: **A rapid and user-friendly assay to detect the Neutrophil gelatinase-associated lipocalin (NGAL) using up-converting nanoparticles**. *Talanta* 2017, **162**:339-344.
21. Xu H, Dong Y, Guo J, Jiang X, Liu J, Xu S, Wang H: **Monoclonal antibody production and the development of an indirect competitive enzyme-linked immunosorbent assay for screening T-2 toxin in milk**. *Toxicon* 2018, **156**:1-6.
22. Kato M, Sasamori E, Chiba T, Hanyu Y: **Cell activation by CpG ODN leads to improved electrofusion in hybridoma production**. *Journal of immunological methods* 2011, **373**:102-110.
23. Huang X, Aguilar Z, Xu H, Lai W, Xiong Y: **Membrane-based lateral flow immunochromatographic strip with nanoparticles as reporters for detection: A review**. *Biosensors bioelectronics* 2016, **75**:166-180.

24. Weeratna R, Comanita L, Davis H: **CPG ODN allows lower dose of antigen against hepatitis B surface antigen in BALB/c mice**. *Immunology cell biology* 2003, **81**(1):59-62.
25. Tong Q, Chen B, Zhang R, Zuo C: **Standardization of clinical enzyme analysis using frozen human serum pools with values assigned by the International Federation of Clinical Chemistry and Laboratory Medicine reference measurement procedures**. *Scandinavian journal of clinical laboratory investigation* 2018, **78**:74-80.
26. Huang D, Ying H, Jiang D, Liu F, Tian Y, Du C, Zhang L, Pu X: **Rapid and sensitive detection of interleukin-6 in serum via time-resolved lateral flow immunoassay**. *Analytical biochemistry* 2020, **588**:113468.
27. Luther T, Bülow-Anderberg S, Larsson A, Rubertsson S, Lipcsey M, Frithiof R, Hultström M: **COVID-19 patients in intensive care develop predominantly oliguric acute kidney injury**. *Acta anaesthesiologica Scandinavica* 2021, **65**(3):364-372.
28. Abers M, Delmonte O, Ricotta E, Fintzi J, Fink D, de Jesus A, Zarember K, Alehashemi S, Oikonomou V, Desai J: **An immune-based biomarker signature is associated with mortality in COVID-19 patients**. *JCI insight* 2021, **6**(1).
29. Yukird J, Wongtangprasert T, Rangkupan R, Chailapakul O, Pisitkun T, Rodthongkum N: **Label-free immunosensor based on graphene/polyaniline nanocomposite for neutrophil gelatinase-associated lipocalin detection**. *Biosensors bioelectronics* 2017, **87**:249-255.
30. Gong Y, Li M, Wang Y: **Carbon nitride in energy conversion and storage: recent advances and future prospects**. *ChemSusChem* 2015, **8**(6):931-946.
31. Yeo S, Bao D, Seo G, Bui C, Kim D, Anh N, Tien T, Linh N, Sohn H, Chong C *et al*: **Improvement of a rapid diagnostic application of monoclonal antibodies against avian influenza H7 subtype virus using Europium nanoparticles**. *Scientific reports* 2017, **7**(1):7933.
32. Chen E, Xu Y, Ma B, Cui H, Sun C, Zhang M: **MonascusCarboxyl-Functionalized, Europium Nanoparticle-Based Fluorescent Immunochromatographic Assay for Sensitive Detection of Citrinin in Fermented Food**. *Toxins* 2019, **11**(10).
33. Bolignano D, Coppolino G, Campo S, Aloisi C, Nicocia G, Frisina N, Buemi M: **Neutrophil gelatinase-associated lipocalin in patients with autosomal-dominant polycystic kidney disease**. *American journal of nephrology* 2007, **27**(4):373-378.

Figures

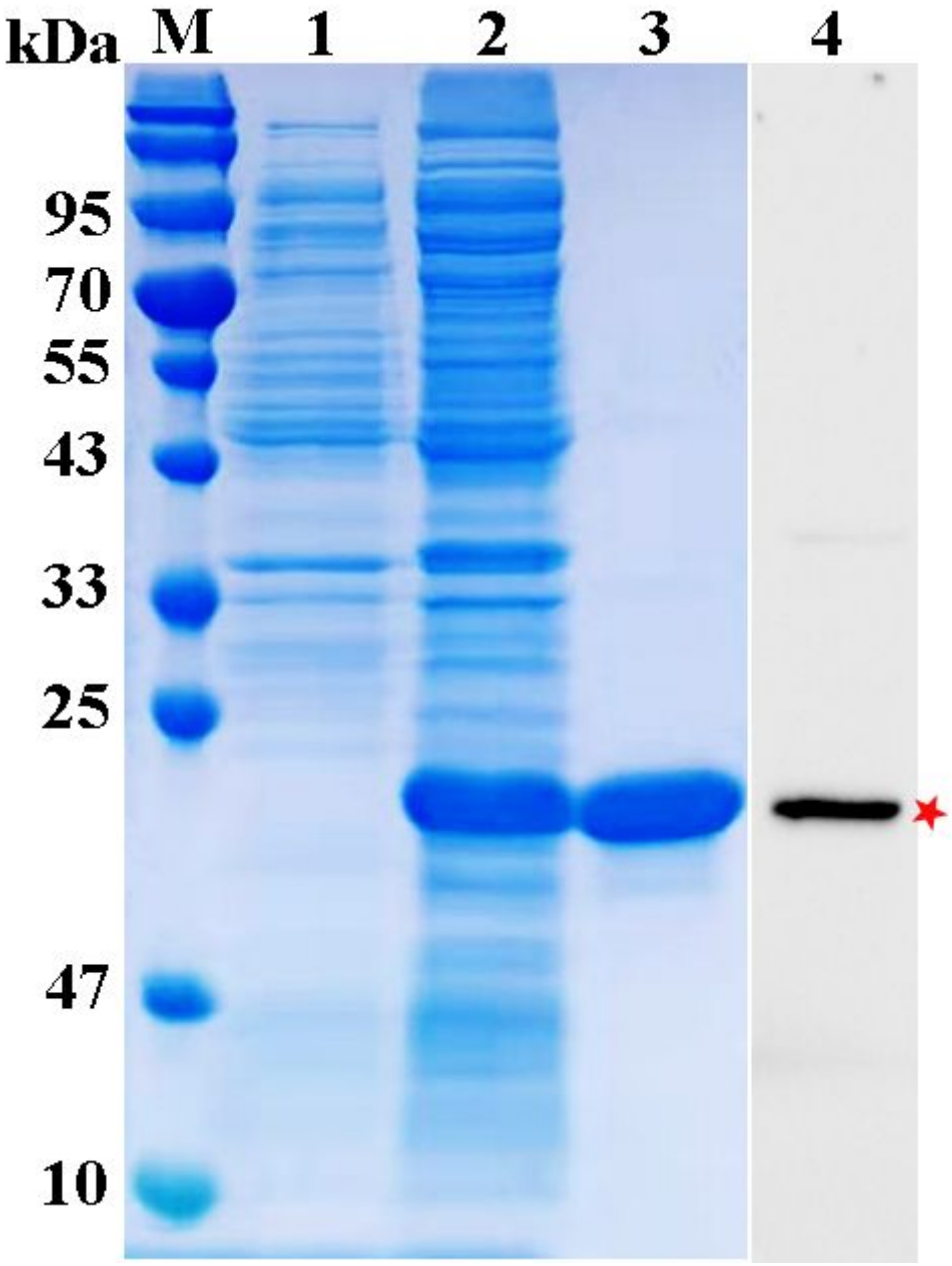


Figure 1

Expression and purification of NGAL-6×His. (A) Purification of NGAL-6×His protein were appraised by SDS-PAGE and Western blot, Lane M, protein marker; Lane 1, supernatant of CHO cell culture; Lane 2, supernatant of induced sample; Lane 3, purified protein ; Lane 4, Western blot analysis of NGAL-6×His expression.

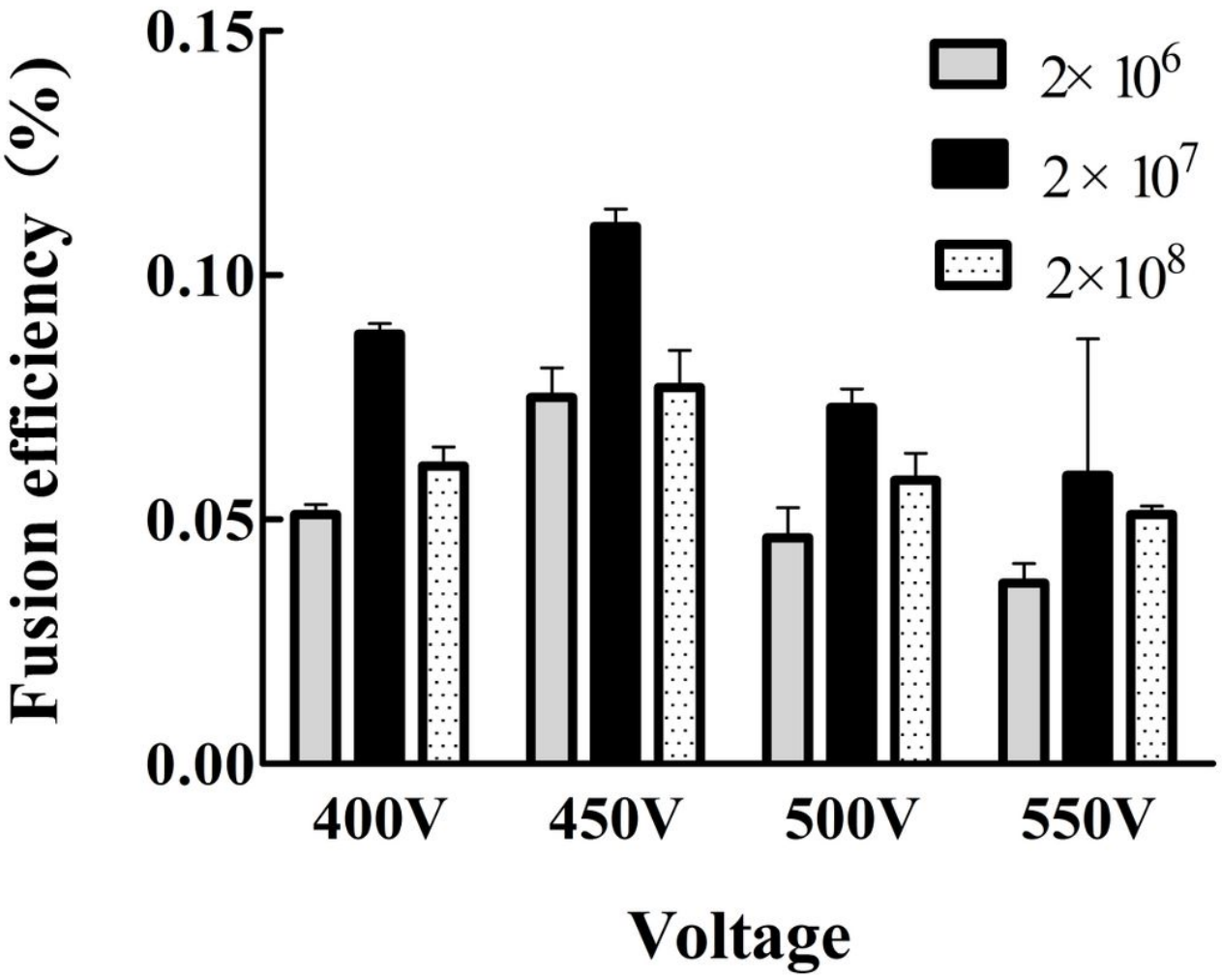


Figure 2

Comparison of fusion efficiency using different the puncture pulse height. The fusion cells were seeded into 96-well plates at approximately 2×105 cells/well. A total of 480 wells was assessed for each condition. The calculated method of the fusion efficiency (%): the total number of colonies in 480 wells were counted,then divided by the number of input B cells and multiplied by 100. The columns represent the average fusion efficiency (%) of 3 experiments, the error bars represent the SD.

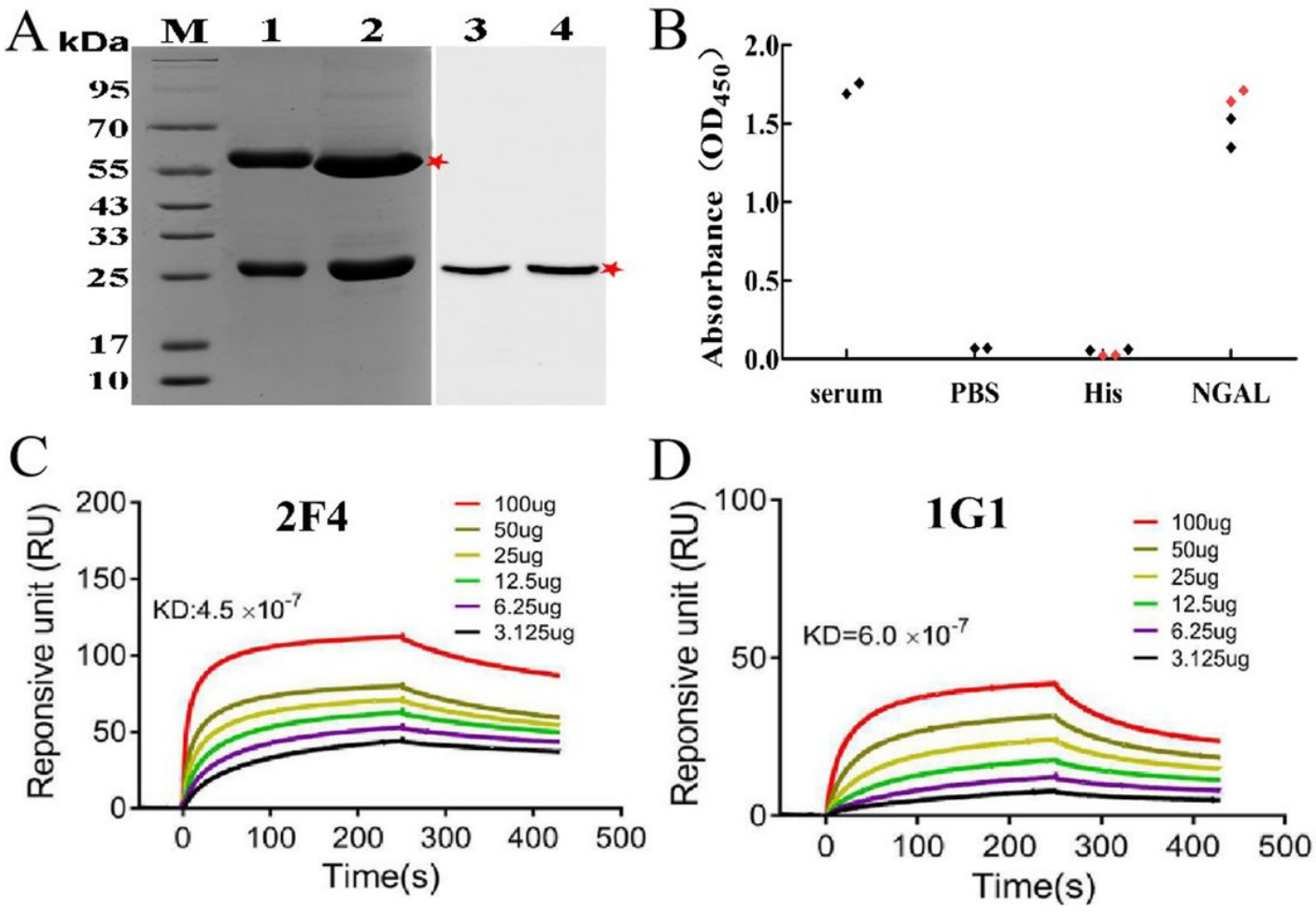


Figure 3

Characterization of monoclonal antibodies. (A) The ascites were assessed by SDS-PAGE and Western blot, Lane M, protein marker; Lane 1, purified the ascites of 2F4; Lane 2, purified the ascites of 1G1; Lane 3, Western blot analysis of mAb 2F4 ; Lane 4, Western blot analysis of mAb 1G1. (B) The Cross-reactivity of 2 hybridoma lines was tested by Indirect ELISA, NGAL-6×His and PCT-6×His were coated onto microtiter plates. The positive control was a NGAL-6×His immune serum; PBS were used as blank control. (C) Relative affinity of MAb 2F4. (D) Relative affinity of MAb 1G1.

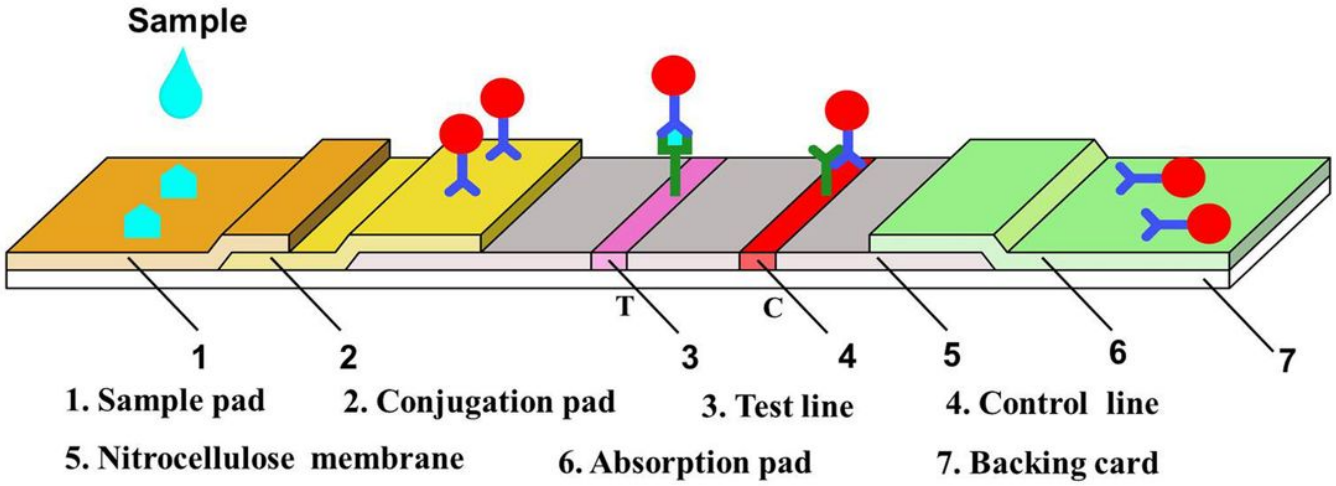
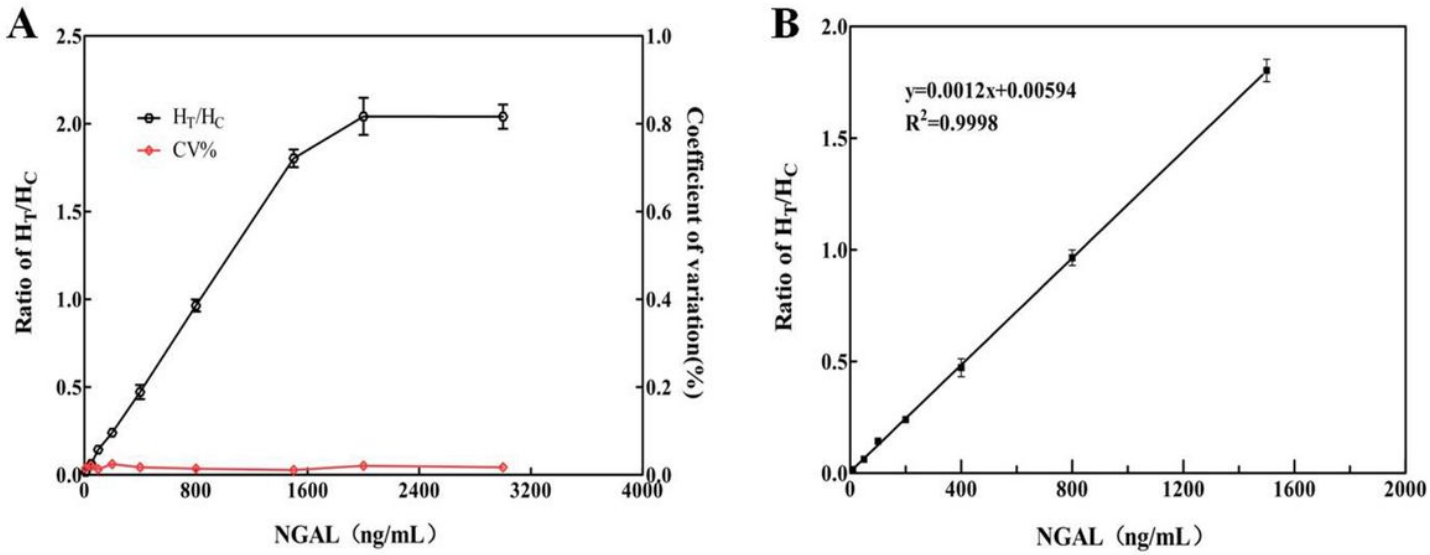


Figure 4

Schematic description of fluorescence immunoassay system employed Europium-conjugated NGAL mAbs.

Figure 5



Standard curves for NGAL. (A) The ratio of HT/HC, by antibody pairing in LFIA, the antibody pairs 2F4-labled1G1 ratio of HT/HC rose with increasing concentration of NGAL. (B) The standard curve of NGAL.

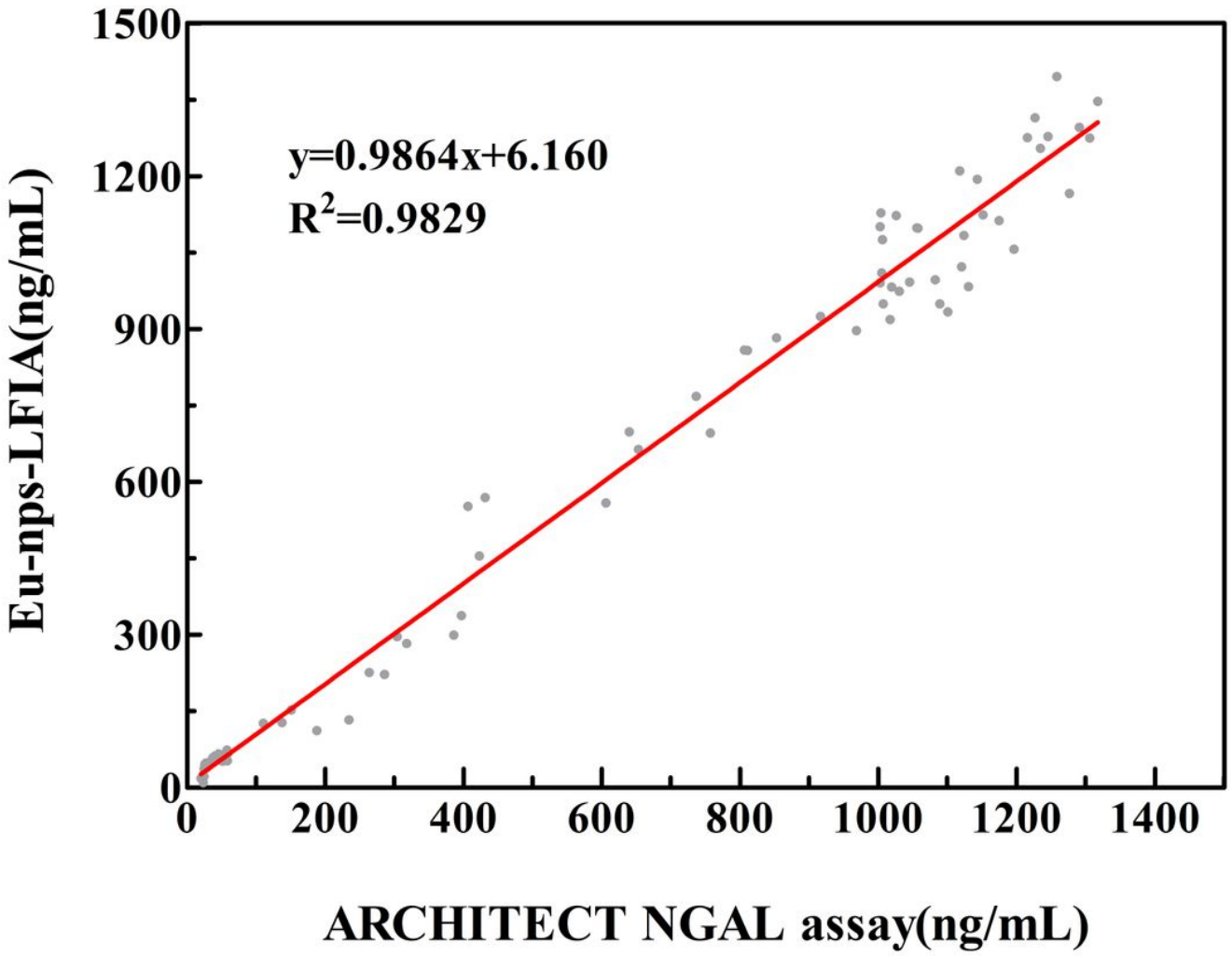


Figure 6

Comparison of EU-NPS-LFIA with ARCHITECT urine assay estimated correlation of the results for NGAL clinical test.