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Development of generic continuous-flow enzyme immunoassay system for analysis of aminoglycosides in serum

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Abstract

A simple generic continuous-flow enzyme immunoassay (CFEIA) for analysis of aminoglycosides in serum has been successfully developed. The developed assay employed a specific monoclonal antibody and β-galactosidase (β-GAL) enzyme as label. The assay involves an off-line competitive binding reaction between the analyte and free labelled analyte for the binding sites of the antibody. After equilibrium is reached, the sample was injected into the flow system. The bound antibody complexes with the analyte and the labelled analyte were trapped in a protein G column, while the unbound free labelled analyte was eluted and detected colorimetrically down-stream, after reaction with chlorophenolic red-β-D-galactopyranoside as a substrate for the β-GAL enzyme. The concentration of the analyte in a sample was quantified by its ability to inhibit the binding of the analyte-enzyme conjugate to the antibody, and the signal was directly proportional to the concentration of the analyte in the original sample. The optimum conditions for the developed CFEIA were investigated and applied to the analysis of tobramycin, as a representative example of the aminoglycosides, in serum samples. The detection limit of the assay was 0.06 μg ml^{-1}. The assay showed good precision; the coefficients of variation were 2.49–4.33 and 3.30–6.82% for intra- and inter-assay precision, respectively. Serum matrix constituents and the endogenous compounds did not interfere with the assay. Analytical recovery of spiked tobramycin, in the concentration range between 0.5 and 8.0 μg ml^{-1}, was 101.55 ± 3.14. The assay results correlated well with those obtained by high-performance liquid chromatography ($r = 0.991$). All the obtained results strongly demonstrate that the developed CFEIA is a suitable method for a rapid and reliable analysis of aminoglycosides in serum.

Keywords: Aminoglycoside antibiotics; Tobramycin; Enzyme immunoassay; Continuous flow; Serum

1. Introduction

Aminoglycosides are widely used in the treatment of human and animal diseases caused by aerobic gram-negative bacillary infections [1]. The major concern in aminoglycosides therapy is their low therapeutic index and their oto- and nephro-

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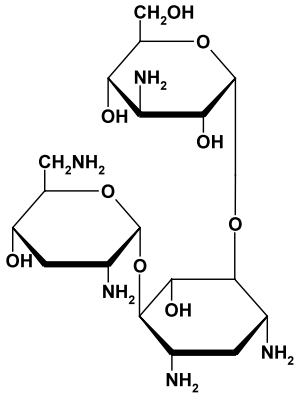
toxicity, the common side effects of all members of aminoglycosides [2]. For safe and effective treatment with aminoglycosides, routine monitoring of their serum concentrations followed by adjustment of the patient dose regimen is required to achieve and maintain the maximum therapeutic efficacy and avoid the side effects toxicity.

Aminoglycosides have been measured by microbiological assay [3–5], high-performance liquid chromatography (HPLC) [6–9], and immunoassay [10–15]. Microbiological assays are semi-quantitative approach, time consuming, and subject to interferences by other antimicrobial drugs. HPLC is a very accurate method, however, separations are difficult to achieve because of the structural similarity between the aminoglycosides members, and the analysis requires derivatization of the sample to improve detectability, or long time for carrying out. Immunoassays involving radioactive labels [10] are extremely sensitive and quite precise, however, the handling of radioactive materials and radioactive waste and high cost are inhibitory factors. Enzyme immunoassays have become the most universal assay method for aminoglycosides because of its simplicity, rapidity, sensitivity and low cost. One additional advantage of enzyme immunoassays is the possibility of utilizing the enzyme labels in the amplification of the signal, if the signal is not sufficient to give the desired assay sensitivity [12].

The traditional enzyme immunoassays, e.g., microplate-based enzyme-linked immunosorbent assays (ELISA) are not practical for aminoglycosides because they adsorb very poorly or not at all to the solid phase supports [16]. To enhance the adsorption of aminoglycosides to solid-phase supports, different pre-treatment approaches were used. These approaches include: irradiation of the solid-phase with ultra-violet [17], or treatment with alcian blue [16], treatment of the target with carbodiimides [18], or conjugation with protein and subsequent immobilization of the conjugate by passive adsorption [19]. The majority of these approaches are still associated with drawbacks such as long incubation periods, multiple washing and mixing steps, high non-specific binding signals, they are labour intensive and/or expensive. Furthermore, the assays are particularly convenient where only small batches of specimens have to be screened or their automation requires expensive instruments. Considering the drawbacks of performing ELISA for aminoglycosides, there was a growing interest in the development of continuous-flow enzyme immunoassays (CFEIA). CFEIA have some important advantages over the former, e.g., improved precision, easy handling, low cost for automation, and higher sample throughput.

The present work is focused on the developing of a generic CFEIA for aminoglycosides; tobramycin was used as a representative example. The assay is based on off-line competitive binding reaction between tobramycin (analyte) and tobramycin conjugated with β-galactosidase (β-GAL) for a limited number of antibody binding sites. After the equilibrium was reached, the sample was introduced into the flow system. The antibody complexes of both tobramycin and tobramycin-β-GAL conjugate were trapped in a protein G column while the unbound tobramycin-β-GAL conjugate was eluted and monitored by colorimetric detection down-stream after reaction with chlorophenolic red-β-D-galactopyranoside (CPRG) as a substrate for the β-GAL enzyme. The concentration of the analyte in a sample was quantified by its ability to inhibit the binding of the enzyme conjugate to the antibody, and the signal was directly proportional to the concentration of the analyte in the original sample.



2. Experimental section

2.1. Chemicals and reagent solutions

Tobramycin was purchased from Eli Lilly Co. (Indianapolis, IN). Monoclonal antibody against tobramycin was obtained from Fitzgerald Industries International Inc. (Concord, MA). β-GAL enzyme (EC 3.2.1.23, grade III), gentamycin sulfate, kanamycin sulfate, neomycin sulfate, streptomycin sulfate and amikacin sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). CPRG was purchased from Boehringer–Mannheim Biochemicals (Indianapolis, IN). *m*-Maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) was obtained from Pierce Chemical Co. (Rokford, IL). Phosphate buffer saline (PBS, 137 mM NaCl, 3 mM KCl and 10 mM sodium phosphate buffer, pH 7.4) was prepared by dissolving 14.3 g $Na_2HPO_4 \cdot 2H_2O$ (Merck, NY), 3.43 g KH_2PO_4 (Sigma Chemical Co., St. Louis, MO), 80 g NaCl (Sigma), 2 g KCl (Sigma) in 1 l of water, and the pH was adjusted with NaOH or HCl (Fisher Scientific Co., Houston, TX). Carrier solution was PBS. Substrate solution was prepared by dissolving CPRG in PBS to a final concentration of 3 mM. All chemicals were of analytical grade and HPLC grade water was produced by filtration through Nanopure II water purification system (Barnstead/Thermolyne, Dubuque, IA), and used throughout this work.

2.2. Preparation of enzyme conjugate

Tobramycin was conjugated with β-GAL enzyme using MBS reagent according to the method described by Kitagawa and Aikawa [20]. The unconjugated tobramycin was removed from the tobramycin-β-GAL conjugate by buffer exchange using a Centricon-30 filter (Amicon, Inc., Beverly, MA). Protein concentration of the conjugate was determined using BCA reagent (Pierce Chemical Co., Rockford, IL), and the conjugate was characterized in terms of specific activity of β-GAL. The specific activity was 225 units mg^{-1} protein; unit was defined as the amount of β-GAL enzyme that converts 1 μmol of CPRG in 1 min.

2.3. Instrumentation

The flow system consisted of a high-pressure pump (model 510, Waters, Milford, MA) was continuously pumping a carrier solution through the system. The samples were mixed and incubated with the antibody and then the solutions were injected via a 50-μl injection loop into the carrier solution stream by an autosampler with a Rheodyne six port injection valve (model 7010, Berkeley, CA). The sampler was controlled by the software provided with the instrument. A column (0.2×2.5 cm) packed with protein G immobilized on Sepharose (Pharmacia Biotech, NJ) was connected to the system after the injection valve. After the protein G column, the enzyme substrate solution was pumped into the system by a second high-pressure pump (model 510, Waters, Milford, MA) via a T-mixer. A merging point located after the protein G column allows the main stream to be mixed (1:1) with the enzyme substrate solution in a knitted Teflon mixing coil (i.d., 0.5 mm), placed into a water bath. The color reaction was developed along the reaction coil. Finally, the signal was detected by an UV–VIS detector (model 204, Spectra-Physics, NY) connected to the system.

2.4. Determination of optimum concentrations of enzyme conjugate and antibody

The optimum concentration of tobramycin-β-GAL conjugate and the best working concentration of antibody were determined by checkerboard immunoassay titration. Purified tobramycin-β-GAL conjugate was diluted into PBS at concentrations of 0.1, 0.25, 0.5, 1, and 2 μg ml^{-1}, and then these solutions were mixed with different concentrations (0.5, 1, 1.5, and 2 μg ml^{-1}) of the antibody, prepared in the same buffer. These solutions were incubated for 15 min, and then introduced into the flow system (Section 2.3). The antibody-bound fraction of the tobramycin-β-GAL conjugate was trapped in a protein G column and the free unbound fraction of the conjugate was eluted and monitored colorimetrically. The obtained signals were plotted as a function of tobramycin-β-GAL conjugate concentration at the various antibody concentrations,

and as a function of antibody concentration at the various tobramycin-β-GAL conjugate concentrations. The optimum antibody concentration was the concentration that yielded 50% binding of the tobramycin-β-GAL conjugate at the concentration used in the assay.

2.5. Continuous-flow enzyme immunoassay procedure

Calibration samples were prepared by mixing the tobramycin standard solution with tobramycin-β-GAL conjugate and antibody in a total volume of 1 ml of PBS, resulting in final concentrations of 1.5 and 0.5 μg ml^{-1} for the antibody and tobramycin-β-GAL conjugate, respectively. After 15 min incubation, the reaction mixtures were injected into the flow system (Section 2.3), and the detector signals were recorded. The data were transformed to a four-parameter curve fit using Slide Write Plus software, version 5.011 (Advanced Graphics Software Inc., CA). For analysis of serum samples, 50 μl serum (after protein precipitation pre-treatment) was dispensed and treated in the same way as the standard solutions. The concentrations of tobramycin in serum samples were obtained by interpolation on the standard curve.

3. Results and discussion

The present study describes a new generic CFEIA for aminoglycosides; tobramycin is used as a representative example. Fig. 1 illustrates the assay setup and the typical signals obtained in the analysis.

3.1. Design and strategy for assay development

Recently competitive immunoassays have been successfully automated using flow injection manifolds incorporating solid-phase reactors to separate the antibody-bound and unbound labeled analyte. Protein A [21], and protein G [22] reactors have been widely used in such assays. Protein G based systems can easily adapted to a different antigen–antibody interactions due to its generic

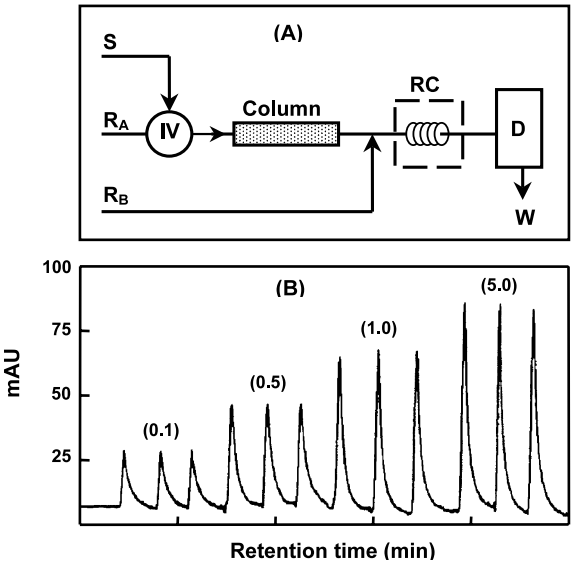


Fig. 1. Schematic diagram for the CFEIA for determination of aminoglycosides (A), and typical detector signals of the analysis (B). Samples were mixed and incubated off-line with antibody and β-GAL enzyme conjugate. During incubation, the analyte in the sample competes with the enzyme conjugate for the binding sites of the antibody. The unbound fraction of the enzyme conjugate is separated on a protein G column, and the eluted amount is determined using the chromogenic substrate, down-stream. S, sample or standard solution; R_A, carrier solution (10 mM PBS); R_B, enzyme substrate solution (3 mM CPRG prepared in the carrier solution); IV, injection valve; RC, reaction coil in a water bath; D, detector; W, waste; mAU, milliabsorbance unit. Figures in parentheses are the tobramycin concentrations in μg ml^{-1}.

recognition and binding to the constant region (Fc region) of the immunoglobulins. Protein G columns showed a good stability and capacity, thus allow analysis of large number of samples before the need for column regeneration [22]. For these reasons, protein G was chosen as a reactor in the study described herein.

The most commonly used enzyme labels are horseradish peroxidase [23–25], alkaline phosphatase [26–28] and β-GAL [29–31]. In principle, any of these enzymes could have been used. β-GAL was chosen for this work because it is absent from human blood, therefore avoid interferences from endogenous enzymes, particularly the proposed assay was designed to use serum samples in the analysis. Furthermore, the optimal enzyme-substrate reaction of β-GAL takes place at pH of 6–8

compared with a pH of 10 for alkaline phosphatase; high pH is not compatible with most antigen–antibody interaction systems.

The chromogenic substrates that are most commonly used for β-GAL are 4-nitrophenyl-β-D-galactopyranoside, resorufine-β-D-galactopyranoside, and CPRG [31]. 4-Nitrophenyl-β-D-galactopyranoside has been nearly exclusively used because it has high turnover rate for the generation of the product (λ_{max} at 405 nm), and consequently gives sensitive assays. Because the assay described herein was designed to measure tobramycin in serum, this substrate is not appropriate because interferences are expected from the endogenous compounds present in the serum matrix that might have absorbing activity at 405 nm. The product of resorufine-β-D-galactopyranoside is purple (λ_{max} at 575 nm), however, it is has a limited solubility in buffer so that concentrations above that required for the maximum velocity of the enzyme-substrate reaction (K_m value) cause flocculation. For this reason, it was not chosen in the system described herein. CPRG is a very sensitive substrate; it gives a purple product (λ_{max} at 575 nm) with excellent solubility in buffer. For these reasons, CPRG was chosen as a substrate for β-GAL enzyme in the system described herein.

In most flow-based enzyme immunoassays, the choice has been to monitor either the antibody-bound fraction of the enzyme conjugate inside the protein G column, or monitoring the eluting free fraction of the label. The former approach is time consuming since four steps are involved for each measurement, i.e. incubation of reagents, separation of the labelled fractions on the column, monitoring of the enzyme conjugate by injection of substrate, and finally regeneration of the protein G column so that the next measurement can be performed. In the second approach, the eluting free fraction of the enzyme conjugate is continuously detected down-stream, therefore, a higher sample throughput can be obtained and the protein G column does not need to be regenerated between the measurements. For these reasons, the present study was designed to monitor the free eluting fraction; the enzyme conjugate. The following sections describe the optimization of different parameters that influence the assay sensitivity and limit of detection as well as application of the optimized system to the analysis of tobramycin in serum samples.

3.2. Choice of enzyme conjugate and antibody concentration

Tobramycin-β-GAL conjugate was prepared by linking the MBS-activated $-CH_2NH_2$ group of tobramycin to the sulfhydryl group of the β-GAL enzyme. The conjugation reaction and the structure of the conjugate are simplified in Fig. 2. The conjugation reaction did not affect the enzyme activity or the immunoreactivity of tobramycin-β-GAL conjugate with the antibody as shown in Fig. 3A. In general, the highest sensitivity of labeled analyte-based competitive enzyme immunoassays is obtained when the lowest possible concentration of the analyte-enzyme conjugate is used. Therefore, it was necessary to optimize the concentration of tobramycin-β-GAL conjugate and the conditions for its detection; time and kinetics of the enzyme-substrate reaction, reaction temperature, and flow rate of the reacting reagents. The optimum concentration of tobramycin-β-GAL conjugate was determined from the titration curve of tobramycin-β-GAL conjugate at varying concentrations of the antibody. The optimum concentration of the antibody that yielded 50% binding of the tobramycin-β-GAL conjugate was 1.5 μg ml^{-1} for all the tested concentrations of the conjugate (Fig. 3B). To select the most appropriate concentration of tobramycin-β-GAL conjugate, competitive assays were performed using 1.5 μg ml^{-1} of the antibody and varying concentrations of the conjugate. The most sensitive assay (lower IC_{50}) was obtained when the conjugate concentration was 0.5 μg ml^{-1} (not shown data). The background signal at these concentrations was 5 mAU. All subsequent experiments were carried out using 1.5 μg ml^{-1} antibody and 0.5 μg ml^{-1} enzyme conjugate.

3.3. Optimization of the detection system

At the optimum concentration of tobramycin-β-GAL, it was desirable to use a concentration of CPRG substrate above that required for max-

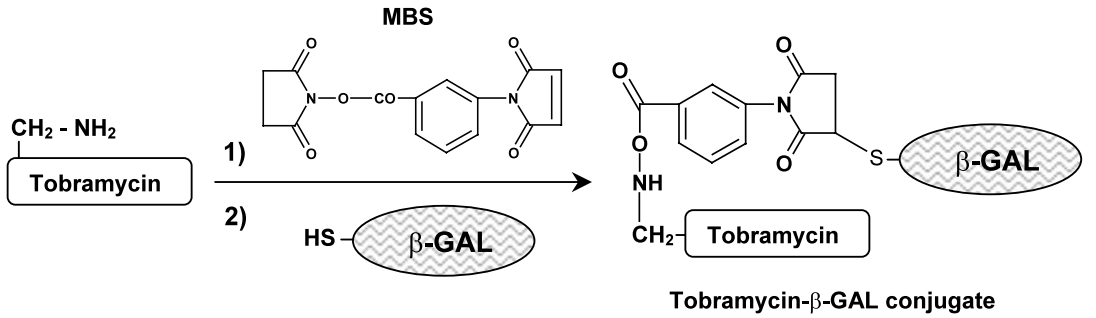


Fig. 2. Preparation of tobramycin-β-GAL conjugate.

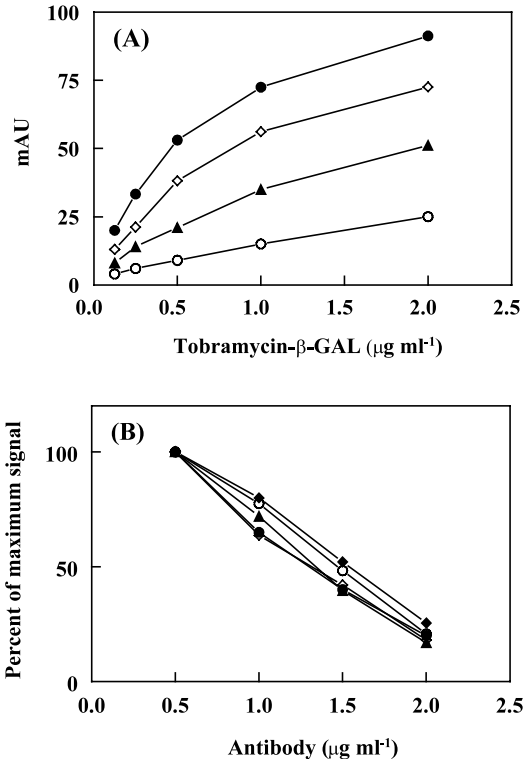


Fig. 3. Checkerboard immunoassay titration for tobramycin-β-GAL conjugate and anti-tobramycin antibody. Varying amounts of the enzyme conjugate were mixed with different amounts of the antibody and incubated off-line. After incubation, the solutions were injected into the flow system, and the absorbance signals were detected. In set A, the signals were plotted as a function of the enzyme conjugate at antibody concentrations of 0.5 (●), 1 (◇), 1.5 (▲), and 2 (○) μg ml^{-1}. In set B, the percent of maximum signals was plotted as a function of antibody concentration at conjugate concentrations of 0.1 (●), 0.25 (◇), 0.5 (▲), 1 (○), and 2 (◆) μg ml^{-1}.

imum velocity of the enzymatic generation the product of CPRG. This concentration of CPRG substrate solution was 1.5 mM after mixing in the substrate flow channel, and thus the working substrate concentration was 3 mM.

Fig. 4 illustrates the effect of enzyme-substrate reaction temperature on the signal. As seen, the highest signal was obtained when the temperature was between 35 and 40 °C. A 37 °C was selected for all further experiments. Fig. 5 illustrates the results of signal dependence on the flow rate. The optimal total flow rate was 1 ml min^{-1}, and it was used for all further experiments.

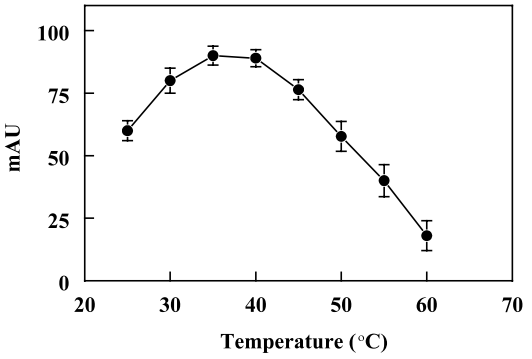


Fig. 4. Effect of temperature on the sensitivity of the detection system for the continuous flow enzyme immunoassay. Reaction mixtures containing 0.5 μg ml^{-1} tobramycin-β-GAL conjugate and 1.5 μg ml^{-1} antibody were allowed to incubate for 15 min, and then introduced into the flow system. The water bath for the enzyme substrate reaction coil was set at the indicated temperature. The values plotted are mean ± S.D. of three determinations.

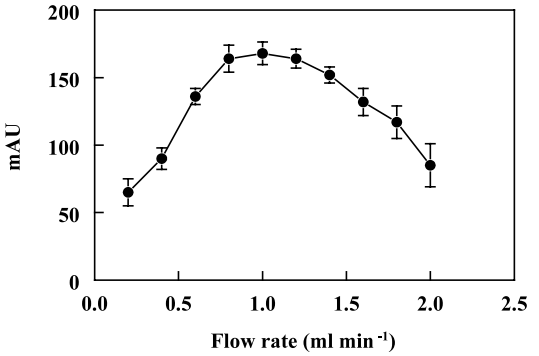


Fig. 5. Effect of flow rate on the sensitivity of the detection system for the CFEIA. Reaction mixtures containing 0.5 μg ml^{-1} tobramycin-β-GAL conjugate and 1.5 μg ml^{-1} antibody were allowed to incubate for 15 min, and then introduced into the flow system. The water bath for the enzyme substrate reaction coil was set at 37 °C. The values plotted are mean ± S.D. of three determinations.

3.4. Assay validation

The calibration curve of the CFEIA for determination of tobramycin in serum is shown in Fig. 6. The calibration samples were prepared by adding a standard tobramycin at concentrations from 0.0 to 400 μg ml^{-1} to PBS, and the samples were analyzed as described in the Section 2. The data showed good correlation on the curve fitting equation; the correlation coefficient (r) was 0.995. The sensitivity of the assay was determined by identifying the limit of detection, defined as the lowest measurable concentration of tobramycin that could be distinguishable from zero concentration ±3 S.D. On the basis of 5 replicate measurements, the limit of detection was 0.06 μg ml^{-1} of tobramycin. In general, the sensitivity of flow-based assays can usually not compete with that of microplate-based assays, however the sensitivity obtained from the method described herein is quite enough for determination of tobramycin in serum without any sample pre-concentration procedure.

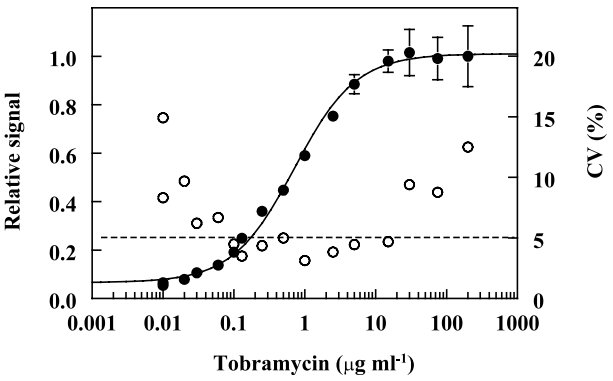


Fig. 6. Calibration curve (●) and precision profile (○) of the CFEIA for tobramycin. Varying concentrations of standard tobramycin were mixed with tobramycin-β-GAL conjugate (0.5 μg ml^{-1}) and antibody (1.5 μg ml^{-1}). The reaction mixtures were further manipulated as described in Section 2. The values plotted are mean ± S.D. of three determinations.

The assay precision profile obtained from the results of calibration standard samples is shown in Fig. 6. The assay gave good results; the coefficients of variation (CV) over the entire linear range of the assay (0.1–10 μg ml^{-1}) were less 5%. In another separate experiments, the intra- and inter-assay precisions were determined at different clinically relevant levels (from pre-dose to post dose) of tobramycin concentrations (0.25–8.0 μg ml^{-1}). The intra-assay precision was assessed by analyzing 6 replicates of each sample in a single run and the inter-assay precision was assessed by analyzing the same sample, as duplicates, in three separate runs on 3 consecutive days. The assay gave satisfactory results over all the tested levels of concentration; the CV values were 2.49–4.33 and 3.30–6.82% for intra- and inter-assay precision, respectively (Table 1). The dilution linearity of the assay response was tested by analyzing a serum sample spiked with 10 μg ml^{-1} tobramycin and serially diluted. A good linear relationship ($Y = 0.056 + 0.932X$, $r = 0.996$) was found between the theoretical amounts of tobramycin and the amounts found by the assay (Fig. 7).

3.5. Interferences studies

In this system, the serum matrix elutes at the same time as the eluting tobramycin-β-GAL conjugate. Therefore, matrix interferences can be expected. To study the matrix effect and potential interferences from endogenous compounds, two series of known amounts of tobramycin were prepared; one in PBS and the other in tobramycin-free serum. The amounts of tobramycin were analyzed by the proposed CFEIA system, and the determined concentrations were treated to calcu-

Table 1
Precision of CFEIA for tobramycin at different concentration levels

Tobramycin (μg ml^{-1})	Intra-assay		Inter-assay	
	Mean ± S.D.[a] (μg ml^{-1})	CV[b] (%)	Mean ± S.D. (μg ml^{-1})	CV (%)
0.25	0.24 ± 0.01	4.33	0.22 ± 0.02	6.82
0.5	0.52 ± 0.02	3.69	0.49 ± 0.03	6.12
1.0	1.04 ± 0.03	2.73	1.09 ± 0.04	3.67
2.0	1.85 ± 0.05	2.49	2.12 ± 0.07	3.30
4.0	5.42 ± 0.15	2.77	4.86 ± 0.21	4.32
8.0	7.82 ± 0.32	4.06	7.94 ± 0.42	5.29

[a] Number of determinations was 6.
[b] CV is the coefficient of variation.

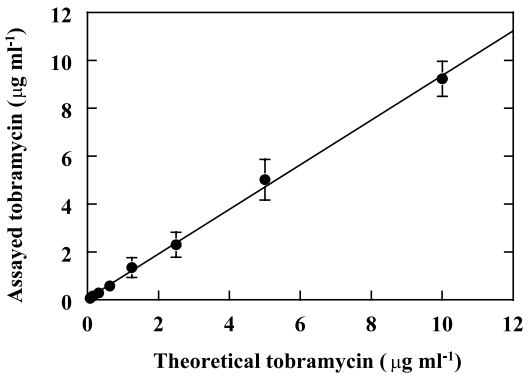


Fig. 7. Dilution test of the CFEIA for tobramycin in serum. A serum sample spiked with tobramycin to a final concentration of 10 μg ml^{-1} was diluted and then analyzed as described in Section 2. The assayed tobramycin concentrations were compared with the theoretical concentrations. Values are mean ± S.D. of triplicate determinations.

late the resulting % recovery according to the following equation:

% recovery
= (serum sample concentration/buffer sample concentration) × 100.

As shown in Table 2, the calculated recovery ranged from 97.23 ± 3.80 to 106.06 ± 10.49%. This indicated the accuracy of the method, as well as the absence of interferences from serum matrix constituents, or the endogenous interfering compounds. The interferences from various aminoglycoside antibiotics (gentamycin, kanamycin, neomycin, streptomycin, and amikacin) at concentrations considerably higher than that may be present in patient serum [32] was studied. No interference was detected from any of these compounds.

Table 2
Recovery studies of tobramycin by CFEIA

Added tobramycin (μg ml^{-1})	Found tobramycin (μg ml^{-1})		Recovery (%) [a]
	In buffer	In serum	
0.5	0.49 ± 0.03 [b]	0.52 ± 0.05	106.06 ± 10.49
1.0	1.00 ± 0.06	1.02 ± 0.07	101.21 ± 6.81
2.0	2.03 ± 0.06	1.97 ± 0.08	97.23 ± 3.80
4.0	4.14 ± 0.11	4.19 ± 0.18	101.28 ± 4.43
8.0	7.96 ± 0.35	8.11 ± 0.50	101.97 ± 6.27
	Average		101.55 ± 3.14

[a] Recovery % = (serum concentration/buffer concentration) × 100.
[b] Values are mean of three determinations.

3.6. Comparison of CFEIA with HPLC

To compare the developed CFEIA procedure to other current methods of aminoglycosides analysis [33], a series of unknown tobramycin samples (0.5–8 μg ml^{-1}) were analyzed by CFEIA and HPLC. As shown in Table 3, the limit of detection of HPLC method was less than 1 μg ml^{-1}, since the unknowns containing less than 1 μg ml^{-1} were not detected by HPLC. Correlation analysis was performed to show the agreement between the results obtained from the two methods. The values obtained by both methods are very close to each

Table 3
Comparison of CFEIA with HPLC for analysis of serum samples spiked with tobramycin

Spiked tobramycin (μg ml^{-1})	Found tobramycin (μg ml^{-1})	
	CFEIA	HPLC
0.5	0.62±0.04[a]	ND [b]
0.63	0.75±0.06	ND
1.0	1.12±0.07	0.95±0.03
1.25	1.62±0.10	1.23±0.03
2.0	2.39±0.14	1.82±0.07
2.5	2.14±0.16	2.46±0.05
4.0	4.05±0.15	4.23±0.04
5.0	5.62±0.19	5.02±0.06
8.0	8.48±0.25	8.23±0.09

[a] Values are mean of triplicate determinations±S.D.
[b] Not detected.

other, and the regression analysis of the results showed a good correlation between the two methods ($Y = -0.134 + 0.979X$, $r = 0.991$). This indicates that the CFEIA is a reliable system for determination of tobramycin in serum. Statistical calculation for the t-test was performed to see if there any difference from zero of the mean results from each method. The calculated t-value at 95% confidence level was found 1.58, which was lower than the tabulated values (1.94 and 2.45 for the one-tail and two-tail, respectively). This indicated that there was any significant difference in the zero of the mean results from each method.

4. Conclusions

A new generic CFEIA system using β-GAL as the enzyme label in combination with colorimetric detection has been successfully developed and validated. The optimized CFEIA system was applied to the analysis of tobramycin; a representative compound for aminoglycosides. This system can be adoptable for measuring other aminoglycosides when the appropriate immunoreagents (specific antibody and proper label) are used. The application of the developed system to the analysis of tobramycin in serum was characterized by a high degree of precision, and accuracy. No preconcentration and reconstitution of serum sample were necessary. The assay described in this report was not subject to interferences from serum matrix or other aminoglycosides at concentrations considerably higher than that may be present in patient serum. The proposed CFEIA system may be easily installed in routine analytical laboratories. In conclusion, the developed CFEIA system is a promising screening system for analysis of aminoglycosides with the elimination of drawbacks of microplate-based immunoassays.

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