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ORIGINAL ARTICLE

Establishment of norvancomycin fluorescence polarization immunoassay for therapeutic drug monitoring

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To establish a rapid and simple fluorescence polarization immunoassay method for determination of norvancomycin serum concentration, we collected 300 serum samples from the patients receiving norvancomycin in the hospitals localized in Shanghai, China. The drug concentrations were measured by the established HPLC method and FPIA with vancomycin kit. A FPIA algorithm for the determination of norvancomycin concentration was established according to the correlation between the FPIA and HPLC results. The methods and algorithm were validated in another 70 clinical samples. HPLC determination showed a good linear correlation within the range of 0.5–100 mg l^{-1} of norvancomycin concentrations. The method was validated via extraction recovery, intra- and inter-day methodological recovery and stability of norvancomycin in serum. Correlation analysis between the measurements of HPLC and FPIA in 300 serum samples gave the linear regression equation: (concentration by HPLC)=0.760×(concentration by FPIA)–0.577 ($P<0.001$, $R^2=0.982$). An algorithm was derived from this correlation for measuring the serum norvancomycin concentrations with FPIA. When it was validated in additional 70 serum samples from patients, 'FPIA algorithm' showed good accuracy versus HPLC: 'FPIA algorithm'=0.93 (HPLC)+0.63, $R^2=0.962$, and 94.3% of the results from FPIA algorithm fell within the range of −20%/+20% of HPLC. This algorithm developed in this study can be easily used for determination of norvancomycin using TDx analyzer with vancomycin kit indirectly. It may also be useful for norvancomycin therapeutic drug monitoring.
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Keywords: fluorescence polarization immunoassay (FPIA); HPLC; norvancomycin; therapeutic drug monitoring

INTRODUCTION

Norvancomycin, developed in China, is an analog of glycopeptide antibiotics. It is derived by demethylation at N-terminus of vancomycin. Norvancomycin is similar to vancomycin in terms of antibacterial activity, spectrum and clinical efficacy. Norvancomycin has been widely used via i.v. infusion in China to treat endocarditis, osteomyelitis and other severe infections caused by *Staphylococcus aureus* (including methicillin-resistant strains). However, nephrotoxicity, ototoxicity and narrow therapeutic window have made it necessary to practise therapeutic drug monitoring (TDM) for vancomycin and norvancomycin.[1–3] It was reported that the adverse drug reactions of norvancomycin are similar to vancomycin, such as nephrotoxicity, ototoxicity, rash and itching.[4–10] Nephrotoxicity will result in serious consequences, especially in the patients who are aged, have kidney diseases or receive concomitant medication excreted from kidney such as aminoglycosides.[8,11–14] So, norvancomycin treatment should be supported by TDM and individualized dosing regimen to decrease the occurrence of adverse reactions and improve efficacy.[3,11]

Fluorescence polarization immunoassay (FPIA) has been widely used on TDM procedure of vancomycin both in China and abroad for a long time. It is simple and able to provide useful information rapidly for the adjustment of dosing regimen.[15,16] However, the norvancomycin-specific TDM system has not been established yet since the approval of this antibiotic. Currently, the norvancomycin serum concentrations can be analyzed by HPLC or microbiological method.[17,18] Nevertheless, HPLC is complicated and technically demanding while the microbiological method is time-consuming and inappropriate in situations of concomitant medications. Therefore, both methods are inappropriate for TDM of norvancomycin in clinical practice. In present study, we used both FPIA and established HPLC to measure the serum norvancomycin concentrations in 300 clinical samples. An algorithm (FPIA algorithm)

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for calculating serum norvancomycin concentration was derived from the correlation analysis between FPIA and HPLC results. Subsequently, FPIA algorithm was validated in additional 70 serum samples.

RESULTS

HPLC method and validation

The retention time of norvancomycin was 5.7 min. No impurity peak was observed at the place near the retention time of norvancomycin in chromatograms. The serum calibration curves were linear over the range of 0.5–100 mg l^{-1}. The equation was A=5220C+2270 (A stands for peak area of norvancomycin and C for concentration), R^2=0.9990. The lower limit of quantification (LLOQ) for norvancomycin was 0.5 mg l^{-1}.

The absolute recovery of norvancomycin from serum was assessed by the ratio of the peak areas from norvancomycin buffer solutions to those from serum samples. The assay was repeated six times for each of 1.8, 18 and 90 mg l^{-1} level quality control (QC) samples. The absolute recovery was 88.90 ± 5.96%.

Each norvancomycin level (1.8, 18, and 90 mg l^{-1}) was tested for six times within a day. The intra-day relative recovery ranged from 100.32 to 103.64%. The inter-day precision (RSD, relative standard deviation) was 0.75–2.54%.

The inter-day relative recovery by measurements of samples on six separate days was 99.57–101.78%. The inter-day precision (RSD) was 0.75–2.54%.

The stability study demonstrated that norvancomycin was stable in serum at room temperature up to 24 h. The QC samples were assayed after 0, 3, 6, 9, 12 and 24 h at room temperature. The recovery of the samples of each norvancomycin level at 24 h was 99.78–100.84%. In addition, the recovery at 24 h in the samples that received pretreatment before putting at room temperature for 0, 3, 6, 9, 12 and 24 h was 98.61–100.55%.

Norvancomycin was also stable after three freeze–thaw cycles at −70 °C. The recovery was 102.22–106.65%.

The 300 clinical samples were determined using the validated HPLC method. The determined concentrations ranged from 1.21 to 59.83 mg l^{-1} with the mean value of 15.62 ± 11.23 mg l^{-1}. The median was 12.93 mg l^{-1}.

Norvancomycin determination by FPIA method

Totally 300 clinical samples were analyzed by FPIA method. The norvancomycin levels were 4.02–77.85 mg l^{-1} in 162 peak level samples and 2.17–53.79 mg l^{-1} in 138 trough samples. Meanwhile, all the samples were also assayed by HPLC. The concentration ranged from 1.67 to 59.83 mg l^{-1} for peak samples and 1.21 to 40.99 mg l^{-1} for trough samples.

The norvancomycin concentrations assayed by FPIA seemed higher than those from HPLC, but analyses illustrated that there is a good linear correlation between the concentrations acquired by these two methods. The regression equation is Y=0.760X–0.577, R^2=0.982 (Figure 1), where X is the concentrations determined by FPIA. Y stands for the value given by HPLC. The 95% confidence interval (CI) of slope was 0.749–0.772. The 95% CI of intercept was −0.875 to −0.279. This analysis suggests that the FPIA method can be used for estimation of the serum concentration of norvancomycin. Here, we named this method as 'FPIA algorithm'.

Validation and application of 'FPIA algorithm'

The norvancomycin concentration in the spiked serum samples derived from 'FPIA algorithm' was linear within 0–60 mg l^{-1} and nonlinear when the concentration was higher than 60 mg l^{-1}. The intra-day RSD of QC samples of low, medium and high level of norvancomycin was lower than 6.08%. The inter-day RSD was lower than 4.75. The method recovery was 87.74–114.34%.

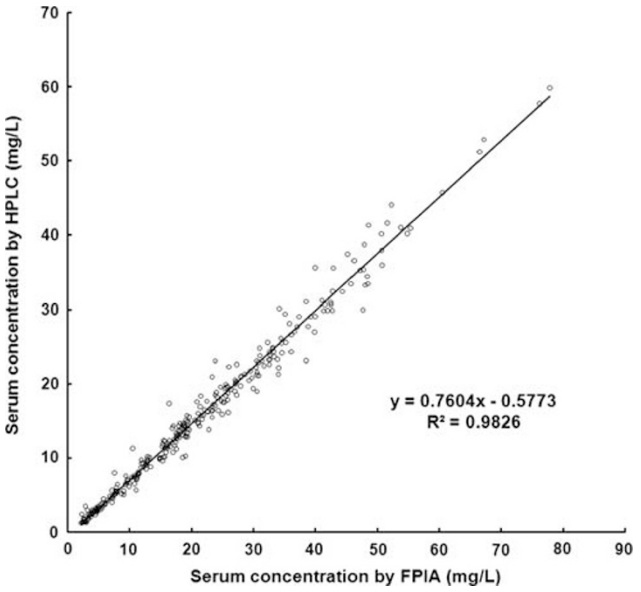

Figure 1 Regression of the concentrations in 300 samples assayed by HPLC versus fluorescence polarization immunoassay (FPIA). This regression established the FPIA algorithm of norvancomycin concentration.

Each of the serum samples containing 50 mg l^{-1} of penicillin, amoxicillin, ampicillin, oxacillin, cefaclor, ceftazidime, imipenem, gentamicin, amikacin, ciprofloxacin or fosfomycin and blank samples were determined by 'FPIA algorithm' and no interference was identified.

The serum samples from eight patients who received norvancomycin concomitantly with another antibiotic, including cefepime, ceftriaxone, cefoperazone-sulbactam, levofloxacin, moxifloxacin, amikacin, or itraconazole, were measured by 'FPIA algorithm' and no interference was found.

Additional 70 TDM samples from patients (male/female, 38/32) were assessed by both HPLC and 'FPIA algorithm' to evaluate the accuracy of 'FPIA algorithm'. The ages of these patients were 57.3 ± 20.6 years old. The body weight was 61.7 ± 11.9 kg. Most of the patients had infectious diseases, tumor, kidney or heart failure or other diseases. The concomitant medications included antimicrobial agents, such as rifampicin, cephalosporins, fluconazole, phosphomycin, metronidazole, imipenem, amikacin and gatifloxacin.

The results of HPLC measurements and FPIA algorithm are summarized in Table 1. The geometric ratio of FPIA algorithm versus HPLC measurements is 0.97 with 90% CI of 0.938–1.002 (P=0.1235). FPIA results showed that 94.3% (n=66) of the results from FPIA achieved the acceptable criteria, which is defined as the discrepancy of serum norvancomycin concentration within ± 20% (concentration by HPLC >5 mg l^{-1}) or ± 1 mg l^{-1} (concentration by HPLC $\leqslant 5$ mg l^{-1}) from true (HPLC) concentration (Table 1). The mean percent absolute relative error was 11.1% (95% CI: 8.16%, 13.99%). The regression equation of the two methods is y (FPIA algorithm)=0.93x (HPLC)+0.63, R^2=0.962 (Figure 2). The 95% CI for the predicted true (HPLC) concentrations corresponding to 5, 10 and 40 mg l^{-1} from FPIA algorithm were 4.16–5.25, 9.54–10.83 and 41.81–42.95 mg l^{-1}, respectively. Similarly, the 95% CI for pre-

Table 1 Summary of results of FPIA algorithm versus HPLC in measurement of serum norvancomycin (*N*=70)

	HPLC (mg l^{-1})	*FPIA algorithm (mg l^{-1})*
Mean (s.d.)	15.63 (11.336)	15.15 (10.737)
Median	15.58	14.10
Minimum, maximum	1.01, 47.49	1.13, 40.39
(FPIA algorithm)/HPLC		
Geometric mean	0.97	
90% CI	0.938, 1.002	
P-value	0.1236	
Number within ±20%[a]	66	
Proportion within ±20% (95% CI)	94.3 (86.01%, 98.42%)	

Abbreviations: CI, confidence interval; FPIA, fluorescence polarization immunoassay.
[a]±20% was defined as the discrepancy of FPIA within ±1 mg l^{-1} (⩽5 mg l^{-1} of HPLC concentration) and ±20% (HPLC concentration >5 mg l^{-1}) of HPLC concentration.

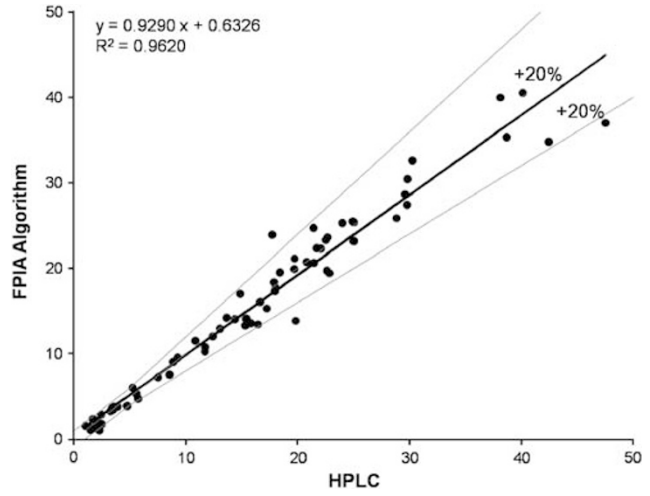

Figure 2 Linear regression of norvancomycin concentrations based on the results of 70 clinical samples derived from 'fluorescence polarization immunoassay algorithm' and HPLC method. Most concentration points are within ±20% of HPLC results.

dicted FPIA algorithm concentrations also contained or closed to 5, 10 and 40 mg l^{-1} of HPLC ponits (Table 2). As shown in Figure 2, most measured points were within ±20% (or ±1 mg l^{-1}) of HPLC (true) value, which illustrated a good accuarcy of 'FPIA alogrithm' against HPLC.

DISCUSSION

In the present study, a reliable and reproducible HPLC method for determination of norvancomycin concentration in serum was constructed first and then was applied on 370 clinical samples for norvancomycin TDM in our hospital. The data revealed that among the collected 'trough concentration samples', only 24% have the true trough concentration of 5–10 mg l^{-1}. British National Formula TDM guidance states that the reference range of vancomycin trough concentration has been changed from 5–10 to 10–20 mg l^{-1}. It is recommended that monitoring trough concentration is sufficient.

Table 2 The correlation of norvancomycin concentration predicted by HPLC or FPIA algorithm at three serum levels

Norvancomycin points (mg l^{-1})	*FPIA algorithm (mg l^{-1}) predicted by HPLC (95% CI)*	*HPLC (mg l^{-1}) predicted by FPIA algorithm (95% CI)*
5	5.3 (4.771, 5.783)	4.7 (4.157, 5.247)
10	9.9 (9.418, 10.425)	10.1 (9.543, 10.627)
40	37.8 (37.267, 38.311)	42.4 (41.814, 42.947)

Abbreviations: CI, confidence interval; FPIA, fluorescence polarization immunoassay.

The low serum concentration might lead to unsatisfactory clinical efficacy, whereas high serum concentration might increase the risk of adverse events. Therefore, it is urgent to establish a rapid, simple and feasible method for clinical TDM on norvancomycin.

FPIA for vancomycin has shown simple and rapid features and widely applied in TDM. There has been the commercial channel to obtain the relevant kit. However, the norvancomycin-specific testing kit is not available yet. Because norvancomycin is a similar chemical substance, which is derived by demethylation on the N-terminus of vancomycin, this study tried to establish the norvancomycin FPIA algorithm based on vancomycin FPIA, assuming that the antibodies of vancomycin could bind to norvancomycin in nonspecific manner and produce a fluorescent response. In 2003, we established the method to determine the norvancomycin concentration using vancomycin kit against the results from microbiological assays. The derived algorithm was: norvancomycin concentration (microbiological method)=0.7534 (FPIA)−0.5948 (R^2=0.9703).[19] This algorithm is similar to that obtained from this study: (HPLC)=0.760 (FPIA)−0.577 (R^2=0.982), but the algorithm based on microbiological assay could be less accurate than that based on HPLC. The drug concentration derived from microbiological assay is the sum of all the components with antibacterial activity instead of norvancomycin alone. In contrast, HPLC can separate the components in serum and determine the concentration of target drug.

The new 'FPIA algorithm' established in this study was based on the results of vancomycin FPIA versus HPLC. The specificity and accuracy of this algorithm was successfully validated. In all the spiked serum samples, the absolute discrepancy fell within ±15% of the results from HPLC. The mean geometric ratio of FPIA algorithm versus HPLC measurements is 0.97 with 90% CI of 0.938–1.002, suggesting 'equivalence' between these two methods. Although the proportion (94.3%) of the results falling within the acceptable range seems less than that defined in the FDA guideline (95%), it does not have substantial impact on the agreement conclusion as the sample size might be less to detect 95% of the results achieving the acceptable criteria. The correlation and regression analyses illustrated a good linear relationship of FPIA algorithm against HPLC and the accuracy of prediction of true concentration with FPIA algorithm further: on the three norvancomycin concentration points, 5, 10 and 40 mg l^{-1}, the 95% CIs for the true or HPLC measured concentration were 4.16–5.25, 9.54–10.83 and 41.81–42.95 mg l^{-1}, respectively. The slightly greater discrepancy at high concentration does not produce any impact on clinical TMD for the safety risk is associated with trough concentration.

Some limitations may be associated with this FPIA algorithm. For samples with low-level norvancomycin, the calculated concentration may be not so accurate. If the serum level of norvancomycin is too low, it may be undetectable by this method. However, in clinical

practice, it is acceptable for TDM to provide only a probable range of drug concentrations, for example, trough concentration $<5\,mg\,l^{-1}$ indicates that it is necessary to adjust doses; while trough level of 10 or $15\,mg\,l^{-1}$ means the dosing regimen is appropriate. Furthermore, at present, norvancomycin is available and used in China only. So the application of this approach may be limited in mainland China alone. In addition, relevant guidelines released by other countries except China do not give the recommended ranges of norvancomycin TDM, although norvancomycin is similar to vancomycin in efficacy, antibacterial spectrum and adverse event profile. The data and evidence we used in this analysis are from both vancomycin and norvancomycin studies. This may make the people question the applicability of this algorithm. This question may be further resolved in actual clinical practice.

CONCLUSION

An 'FPIA algorithm' was successfully established in this study. It takes the advantage of the available vancomycin kit and TDx to indirectly get the norvancomycin concentrations in serum. The turnaround time of this method is within 20 min. Only 1–2 ml blood sample is required for this test. The result is not affected by concomitant use of other antibiotics. It is easy to operate, rapid, accurate and specific. Its application in norvancomycin TDM is helpful for individualized dosing regimen so as to reduce norvancomycin toxicity and improve clinical efficacy. We will further validate this algorithm in real world clinical practice.

METHODS

Blood samples

Blood samples for norvancomycin TDM were collected from the hospitals in Shanghai from 2003 to 2005. The 370 blood samples included 198 for norvancomycin peak levels and 172 for trough levels. The peak samples were collected at 30 min to 1 h after the end of i.v. infusion. The trough samples were collected immediately before administration. All the samples were collected at steady state.

HPLC system for determination of norvancomycin concentration

The Waters Alliance 2695 HPLC system was equipped with Waters 2487 UV detector at 229 nm of wavelength, and Waters Atlantis dC18 ($150\times4.6\,mm^2$; ID, 5 μm) chromatographic column (30 °C; Waters, Milford, MA, USA). The mobile phase was composed of 0.05 mol ammonium acetate (pH 2.9) and acetonitrile (90:10, V/V). Flow rate was 1.0 ml per minute.

Preparation of stock solution, calibration curve and QC samples

Appropriate amount of norvancomycin standard (83.4%. purity, Lot No. 000627, National Institutes for Food and Drug Control, China) was dissolved in buffer (pH 6) to get the stock solution with final concentration of $4000\,mg\,l^{-1}$. The stock solution was then diluted to a series of buffer solutions ($5–1000\,mg\,l^{-1}$). Norvancomycin serum calibration curve was established by diluting the above buffer solutions with blank human serum to final concentrations of 0.5, 1, 5, 10, 25, 50 and $100\,mg\,l^{-1}$, respectively.

Norvancomycin standard was reconstituted with buffer (pH 6) to get the QC stock solution of $3000\,mg\,l^{-1}$, which was further diluted to 5, 18, 180 and $900\,mg\,l^{-1}$ with buffer. Final QC samples containing 0.5, 1.8, 18, $90\,mg\,l^{-1}$ norvancomycin were prepared by diluting the above buffer solutions with blank human serum.

Pretreatment of blood samples

Acetonitrile (0.2 ml) was added into 0.2 ml blood sample. Vortex it to mix well, let the solution stand for 10 min. The mixture was then centrifuged at 7800 *g* for 10 min. The supernatant (0.2 ml) was mixed with 0.4 ml mobile phase. Twenty microliters of the supernatant was injected for HPLC analysis.

Method validation

The validation of HPLC method included extraction recovery, specificity, intra- and inter-day variability, stability of blood samples at room temperature and stability of freeze–thaw cycles.

The validated HPLC method was used to establish 'FPIA algorithm' by correlation analysis of the norvancomycin assay results of 300 blood samples and validation of 'FPIA algorithm' with additional 70 samples.

Establishment of FPIA algorithm

TDx Automated Fluorescence Polarization Analyzer (Abbott Laboratories, Abbott Park, IL, USA) and vancomycin TDx kit were used to measure the concentration of norvancomycin. Vancomycin calibration curve (0.0–$100.0\,mg\,l^{-1}$) and vancomycin QC (7, 35, $75\,mg\,l^{-1}$) were commercially provided by Abbott Pharmaceutical Co. Ltd.

The blood samples (n=300) including 162 peak level samples and 138 trough level samples were assayed by TDx automated fluorescence polarization analyzer with vancomycin TDx kit. Vancomycin QC samples were used for inter-run QC. Norvancomycin concentration in all the samples was also assayed by the established HPLC method. A linear regression equation was derived from the correlation analysis between the results determined by the two methods. This algorithm can be used to estimate serum norvancomycin concentration indirectly using TDx analyzer with vancomycin TDx kit.

FPIA method validation

Accuracy and precision of 'FPIA algorithm'. Norvancomycin calibration curve ($0–75\,mg\,l^{-1}$) and QC samples (60, 35, $7\,mg\,l^{-1}$) in serum were prepared and assayed with TDx method. Because the true concentration of norvancomycin is lower than the concentration determined by TDx, the upper limit of norvancomycin calibration curve and high QC we used were lower than vancomycin to avoid concentration result out of range. The QC samples of various concentrations were assayed. Each test was repeated five times on the same day for intra-day, and repeated three times on different days for inter-day accuracy and precision evaluation. The TDx QC samples were assayed simultaneously. The concentrations on calibration curve and QC samples were calculated using FPIA algorithm.

Specificity. Serum samples each containing $50\,mg\,l^{-1}$ of penicillin, amoxicillin, ampicillin, oxacillin, cefaclor, ceftazidime, imipenem, gentamicin, amikacin, ciprofloxacin or fosfomycin or $45\,mg\,l^{-1}$ of norvancomycin as well as blank serum samples were assayed by FPIA method to evaluate FPIA specificity. Serum samples from either the patients who received antibiotics other than norvancomycin, or who received monotherapy of norvancomycin, were also analyzed.

Application of FPIA algorithm

Both the 'FPIA algorithm' procedure and HPLC method were used to determine the concentrations in 70 clinical serum samples. The results were compared with assess the accuracy of FPIA algorithm.

To evaluate the accuracy of FPIA algorithm, the primary objective of the validation is the proportion of results from FPIA algorithm falling within the acceptable criteria, which is defined as the discrepancy of results from FPIA algorithm was within ±20% for concentration by HPLC $>5\,mg\,l^{-1}$ or $\pm1\,mg\,l^{-1}$ for concentration by HPLC $\leqslant5\,mg\,l^{-1}$. The significance of discrepancy of FPIA algorithm from HPLC measurement was analyzed using analysis of variance based on log-transformation. The mean geometric ratio of FPIA algorithm to HPLC and corresponding 90% confidence interval was constructed for the equivalence assessment. To assess the extent of discrepancy, the mean absolute relative error was calculated according to the FDA guideline.[20] Meanwhile, the linear regression equation of FPIA algorithm versus HPLC was estimated to assess the discrepancy of lower, middle and upper predicted values (5, 15 and $40\,mg\,l^{-1}$).

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1 Andres, I., Lopez, R., Pou, L., Pinpl, F. & Pascualm, V. Vancomycin monitoring: one or two serum levels? *Ther. Drug. Monit.* **19,** 614–619 (1997).
2 Karam, C. M., McKinnon, P. S., Neuhauser, M. M. & Rybak, M. J. Outcome assessment of minimizing vancomycin monitoring and dosing adjustments. *Pharmacotherapy* **19,** 257–266 (1999).
3 Zhang, J. *et al*. Population pharmacokinetic and pharmacodynamic modeling of norvancomycin. *Eur. J. Clin. Microbiol. Infect. Dis.* **27,** 275–284 (2008).
4 Darko, W., Medicis, J. J. & Smith, A. Mississippi mud no more: cost-effectiveness of pharmacokinetic dosage adjustment of vancomycin to prevent nephrotoxicity. *Pharmacotherapy* **23,** 643–650 (2003).
5 Cantu, T. G., Yamanaka-Yuen, N. A. & Leitman, P. S. Serum vancomycin concentrations: reappraisal of their clinical value. *Clin. Infect. Dis.* **18,** 533–543 (1994).
6 Wilhelm, M. P. & Estes, L. Symposium on antimicrobial agents—part XII. Vancomycin. *Mayo Clinic. Proc.* **74,** 928–935 (1999).
7 Rybak, M. J. & Boike, S. C. Monitoring vancomycin therapy. *Drug Intell. Clin. Pharm.* **20,** 757–761 (1986).
8 Farber, B. F. & Moellering, R. C. Jr Retrospective study of the toxicity of preparations of vancomycin from 1974 to 1981. *Antimicrob. Agents Chemother.* **23,** 138–141 (1983).
9 Mellor, J. A., Kingdom, J., Cafferkey, M. & Keane, C. T. Vancomycin toxicity: a prospective study. *J. Antimicrob. Chemother.* **15,** 773–780 (1985).
10 Sorrell, T. C. & Collignon, P. J. A prospective study of adverse reactions associated with vancomycin therapy. *J. Antimicrob. Chemother.* **16,** 235–241 (1985).
11 Cimino, M. A., Rotstein, C., Slaughter, R. L. & Emrich, L. J. Relationship of serum antibiotic concentrations to nephrotoxicity in cancer patients receiving concurrent aminoglycoside and vancomycin therapy. *Am. J. Med.* **83,** 1091–1097 (1987).
12 Celik, I., Cihangiroglu, M., Ilhan, N., Akpolat, N. & Akbulut, H. H. Protective effects of different antioxidants and amrinone on vancomycin vancomycin-induced nephrotoxicity. *Basic Clin. Pharmacol. Toxicol.* **97,** 325–332 (2005).
13 Le Moyec, L. *et al*. Aminoglycoside and glycopeptide renal toxicity in intensive care patients studied by proton magnetic resonance spectroscopy of urine. *Crit. Care Med.* **30,** 1242–1245 (2002).
14 Nishino, Y. *et al*. Inhibition of vancomycin-induced nephrotoxicity by targeting superoxide dismutase to renal proximal tubule cells in the rat. *Redox. Rep.* **7,** 317–319 (2002).
15 Farin, D., Piva, G. A., Gozlan, I. & Kitzes-Cohen, R. A modified HPLC method for the determination of vancomycin in plasma and tissues and comparison to FPIA (TDX). *J. Pharm. Biomed. Anal.* **18,** 367–372 (1998).
16 Jehl, F., Monteil, H., Gallion, C. & Thierry, R. C. HPLC RIA FPIA. Evaluation of 3 methods for the assay of vancomycin. *Pathol. Biol. (Paris)* **33** (5 Pt 2), 511–516 (1985).
17 Jesús Valle, M. J., López, F. G. & Navarro, A.S. Development and validation of an HPLC method for vancomycin and its application to a pharmacokinetic study. *J. Pharm. Biomed. Anal.* **48,** 835–839 (2008).
18 Najjar, T. A., al-Dhuwailie, A. A. & Tekle, A. Comparison of high-performance liquid chromatography with fluorescence polarization immunoassay for the analysis of vancomycin in patients with chronic renal failure. *J. Chromatogr. B Biomed. Appl.* **672,** 295–299 (1995).
19 Zhang, J. *et al*. Clinical pharmacokinetics and therapeutic drug monitoring of norvancomycin. *Chinese J. Infect. Chemother.* **3,** 202–205 (2003).
20 US Department of Health and Human Services, Food and Drug Administration. Recommendations: Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver applications for manufacturers of *in vitro* diagnostic devices, http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079632.htm (2008).