วารสาร
เทคนิคการแพทย์และกายภาพบำบัด
JOURNAL OF MEDICAL TECHNOLOGY AND PHYSICAL THERAPY

นิพนธ์ต้นฉบับ
ORIGINAL ARTICLE

การตรวจวิเคราะห์ระดับยาไซโคลสปอรินและเอ็ฟเวอโรไลมัสในเลือดผู้ป่วย เปรียบเทียบระหว่างเครื่องวิเคราะห์อัตโนมัติ Cobas e411 และ Indiko Plus

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Received: *July 24, 2018*
Revised: *September 29, 2018*
Accepted: *November 15, 2018*

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบประสิทธิภาพของวิธี electrochemiluminescence immunoassay (ECLIA) ในการตรวจวิเคราะห์ระดับยาไซโคลสปอริน (cyclosporin, CsA) และเอ็ฟเวอโรไลมัส (everolimus, EVL) ด้วยเครื่องวิเคราะห์อัตโนมัติ cobas e411 (Roche Diagnostics) กับวิธี cloned enzyme donor immunoassay (CEDIA) ในการตรวจวิเคราะห์ระดับยาไซโคลสปอริน และวิธี Quantitative Microsphere System (QMS) ในการตรวจวิเคราะห์ระดับยาเอ็ฟเวอโรไลมัสด้วยเครื่องวิเคราะห์อัตโนมัติ Indiko Plus (Thermo Fisher Scientific) โดยทำการตรวจวิเคราะห์ในเลือดของผู้ป่วย ที่ส่งตรวจ ณ ห้องปฏิบัติการพิษวิทยาคลินิก โรงพยาบาลศิริราช จำนวนระดับยาละ 40 ตัวอย่าง แล้วทดสอบความสัมพันธ์ของผลการตรวจวิเคราะห์ระดับยาระหว่างเครื่องวิเคราะห์อัตโนมัติทั้งสอง โดยใช้วิธีทางสถิติ ผลการศึกษาพบว่า เมื่อทดสอบด้วยการตรวจวิเคราะห์สารควบคุมคุณภาพ ในการตรวจวิเคราะห์ระดับยาไซโคลสปอรินและเอ็ฟเวอโรไลมัสด้วยเครื่องวิเคราะห์อัตโนมัติ Cobas e411 มีความเที่ยงอยู่ในเกณฑ์ที่ดี (1.51 – 5.05 %CV) เมื่อทดสอบด้วยการตรวจวิเคราะห์ระดับยาในเลือดของผู้ป่วย ผลที่ได้จากทั้งสองเครื่องมีความสัมพันธ์กันดี ($r > 0.95$, $r^2 \geq 0.90$) มีค่าอคติอยู่ระหว่าง 2.11 – 2.69% สำหรับการตรวจวิเคราะห์ระดับยาไซโคลสปอริน และ 12.29 – 12.61% สำหรับการตรวจวิเคราะห์ระดับยาเอ็ฟเวอโรไลมัสมีค่าความผิดพลาดรวมอยู่ในเกณฑ์ที่ยอมรับได้ จากผลการศึกษาจึงสรุปได้ว่าวิธี ECLIA ที่ใช้ในการตรวจวิเคราะห์ระดับยาไซโคลสปอรินและเอ็ฟเวอโรไลมัสด้วยเครื่องวิเคราะห์อัตโนมัติ Cobas e411 มีประสิทธิภาพดี และสามารถนำมาใช้ทดแทนวิธี CEDIA และ QMS ในการตรวจวิเคราะห์ระดับยาไซโคลสปอรินและยาเอ็ฟเวอโรไลมัสด้วยเครื่องวิเคราะห์อัตโนมัติ Indiko Plus (Thermo Fisher Scientific) ได้

คำสำคัญ: การตรวจติดตามการรักษาด้วยยา, การเปรียบเทียบวิธีวิเคราะห์, เครื่องวิเคราะห์อัตโนมัติ, ไซโคลสปอริน, เอ็ฟเวอโรไลมัส

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ORIGINAL ARTICLE

Determination of cyclosporine and everolimus blood levels compared between the cobas e411 and Indiko Plus automated chemistry analyzers

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Abstract

To compare the efficacy of electrochemiluminescence immunoassay (ECLIA) to measure cyclosporin (CsA) and everolimus (EVL) blood levels on the cobas e411 automated chemistry analyzer (Roche Diagnostics) versus the efficacy of cloned enzyme donor immunoassay (CEDIA) to measure CsA blood level and the Quantitative Microsphere System (QMS) to measure EVL blood level on the Indiko Plus automated chemistry analyzer (Thermo Fisher Scientific). This comparative study included 40 blood samples that were collected for measurement of CsA and 40 blood samples that were collected for measurement of EVL that were analyzed at the Clinical Toxicology Laboratory of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. CsA and EVL blood level results were statistically analyzed and compared between the two automated analyzer. The precision of internal quality control for CsA and EVL measurement using the automated cobas e411 analyzer ranged from 1.51 to 5.05 %CV. The Pearson's correlation coefficient ($r > 0.95$, p-value <0.01) indicates a strong positive correlation. The results of linear regression revealed good correlation between the two compared systems ($r^2 \geq 0.90$). The percentages of bias ranged from 2.11% to 2.69% for CsA, and from 12.29% to 12.61% for EVL. Statistically significant differences were observed between group means, as determined by ANOVA ($p<0.01$). Calculated total errors were within acceptable range for all evaluated parameters. The results of this study revealed that ECLIA on the cobas e411 system demonstrated good analytical performance that correlated well with the results of the CEDIA and QMS assays on the Indiko Plus system. Accordingly, the cobas e411 system should be introduced into routine practice for determination of CsA and EVL blood levels.

Keywords: Therapeutic drug monitoring, Method comparison, Automated analyzer, Cyclosporine, Everolimus

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INTRODUCTION

Cyclosporine (CsA) and Everolimus (EVL) are potent immunosuppressant drugs that are used to prevent the newly transplanted organ rejection and to treat autoimmune diseases.[1, 2] The use of these drugs is restricted by substantial adverse effects and a narrow therapeutic window. As a result of intraindividual and interindividual pharmacokinetics variation, therapeutic monitoring of immunosuppressive drug concentrations is required so as to avoid drug toxicity and to desire therapeutic effect.[2-4]

Automated immunoassays become an important role in a modern routine clinical laboratory for therapeutic drug monitoring. The clinical toxicology laboratory at our center currently uses the two following systems to analyze blood immunosuppressant blood levels: the Indiko™ Plus Clinical and Specialty Chemistry System (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the recently launchedcobas e411 immunoanalyzer (Roche Diagnostics, Risch-Rot-kreuz, Switzerland). The Indiko Plus system features two immunoassays for evaluating immunosuppressant level, including the cloned enzyme donor immunoassay (CEDIA) for determination of CsA level, and the Quantitative Microsphere System (QMS) for determination of EVL level. In contrast, the cobas e441 system employs a newly developed method that uses electrochemiluminescence immunoassay (ECLIA) to evaluate blood levels of both CsA and EVL.

Although the general properties of the two systems are similar, but the sensitivity and the measurement ranges of ECLIA EVL are more than QMS EVL (0.5 – 30 ng/mL for ECLIA EVL, 2.0–20 ng/mL for QMS EVL). Moreover, the reagent of ECLIA CsA and ECLIA EVL assays are stable after opening than the reagent of QMS EVL and CEDIA CsA assay (84 days for ECLIA assay, 60 days for QMS and CEDIA assay). The determination of EVL level on ECLIA assay revealed good precision, accuracy and sensitivity for TDM and generally agreed with LC-MS/MS methods.[5] In contrast, the method comparisons between the QMS method and the LC-MS/MS method demonstrated a significant positive bias.[6, 7] The determination of CsA level on ECLIA and CEDIA are also showed close agreement with LC-MS/MS methods.[7, 8] However, method comparison between the ECLIA and the QMS method has an unclear result.

The aim of this study was to compare the efficacy of ECLIA to measure CsA and EVL blood levels on the cobas e411 automated chemistry analyzer versus the efficacy of CEDIA to measure CsA blood level and QMS to measure EVL blood level on the Indiko Plus automated chemistry analyzer.

MATERIALS AND METHODS

This comparative study included 40 blood samples that were collected for measurement of CsA, and 40 blood samples that were collected for measurement of EVL that were analyzed at the Clinical Toxicology Laboratory of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Siriraj Hospital is Thailand's largest national tertiary referral center. This study was rated as exempt from procedural review, and was approved by the Siriraj Institutional Review Board (SIRB) [COA no. Si 595/2017].

Material

The ECLIA CsA and ECLIA EVL assays were used for measurement of CsA and EVL concentrations on a Roche Diagnostics cobas e411 analyzer. CEDIA CsA for measurement of CsA concentration, and QMS EVL for measurement of EVL concentration were performed on a Thermo Fisher Scientific Indiko Plus analyzer.

Control specimens

Commercially available PreciControl ISD (for Immunosuppressive drugs) and PreciControl Everolimus (Roche Diagnostics) at 3 levels (low, medium, and high) were used. Within-day and between-day precision of the different methods were determined using 3 different levels of control material.

Patient whole blood samples

This study included samples based on ethylenediaminetetraacetic acid (EDTA) whole blood from 40 different patients for each drug for a total of 80 samples. Blood samples were collected from patients who presented at the Clinical Toxicology Laboratory of Siriraj Hospital for immunochemistry analysis of CsA and/or EVL blood concentration. When a sample size of n=20 was used, 5 samples for level lower than therapeutic range, 10 samples for medium level (within therapeutic range), and 5 samples for level higher than therapeutic range were used. When a sample size of n=40 was used, the numbers in each group were 10, 20, and 10 for the low, medium, and high level groups, respectively. The first analysis was performed on the Indiko Plus analyzer, which uses CEDIA CsA assay for determination of CsA, and QMS EVL assay for determination of EVL. The second analysis was performed on the cobas e411 analyzer, which uses ECLIA CsA and EVL assays for determination of CsA and EVL concentrations, respectively. Blood samples were stored at -20°C until analysis.

Method analysis

CEDIA immunoassay

The CEDIA CsA assay is based on β-galactosidase, which is a bacterial enzyme has been genetically designed into two inactive fragments. These fragments spontaneously reassociate to form fully active enzymes that cleave a substrate, which produces a color change that can be measured the photometric intensity. The resultant absorbance change are directly relationship with the drug concentration in the sample.[9] Briefly, 100 µL of patient whole blood was accurately pipetted into the sample cup, and then 400 µL of CEDIA CsA lysing reagent was added. The sample cup was mixed for 5 seconds and then immediately placed into the analyzer.[10]

QMS immunoassay

The QMS EVL assay is a homogeneous particle-enhanced turbidimetric immune-assay. The assay is based on competition between drug coated onto a microparticle and drug in the sample for a fixed number of specific antibody binding sites. Briefly, 300 µL of patient whole blood was accurately pipetted into a microcentrifuge tube. Three hundred and fifty µL of methanol and 50 µL of QMS EVL Precipitation Reagent were then added. The mixture was then vortexed at the highest speed for at least 35 seconds, and then centrifuged for 10 minutes at 14,000 rpm. After centrifuging, the supernatant was transferred into

a sample cup and immediately loaded into the analyzer. [11]

ECLIA immunoassays

The ECLIA CsA and ECLIA EVL immunoassays are based on chemiluminescence reaction of a ruthenium complex and tripropylamine by applying a voltage to the sample solution.[12] Patient whole blood samples are incubated at room temperature and mixed by inverting before use. Three hundred microliters of blood sample and 300 µL of Immuno-Suppressive Drug (ISD) Sample Pretreatment Reagent were pipetted into a microcentrifuge tube. The tube is capped, vortexed for 10 seconds, and centrifuged for 4 minutes at 14,000 rpm. The supernatant is transferred into a sample cup and then immediately loaded into the analyzer.[13, 14]

Statistical analysis

CsA and EVL concentrations were compared using Pearson's correlation coefficient and linear regression analysis. Because of the time needed for analysis and the facilities required, reference methods are not suitable for method comparison. The QMS EVL and CEDIA CsA assays are one of the more recently approved tests for determination of EVL[6, 15] and CsA[16] concentrations in whole blood in organ-transplanted patients. Thus, this study will direct comparison with the definitive method (QMS EVL and CEDIA CsA assays on indiko plus). Analysis of variance (ANOVA) was used for comparison of different patients and methods. Systemic error (SE) was calculated as the percentage of bias using equation 1. Random error (RE) was calculated as the within-day and between-day precision (coefficient of variation, %CV) for quality control (equation 2).[17] After that, the calculated total errors (TE_{cal}) were calculated (equation 3) compare with allowable total error (TE_a) (equation 4), with the calculated total error required to be lower than the TE_a.[18] Statistical analyses were performed using Microsoft Office® Excel Professional Edition (Microsoft Corporation, Redmond, WA, USA) and SPSS Statistics version 19.0 for Windows (SPSS, Inc., Chicago, IL, USA).

To test whether the sample size affects the result of comparison, we first analyzed and based our calculations on 20 samples (low = 5, medium = 10, high = 5), and then we analyzed and based our calculations on 40 samples (low = 10, medium = 20, high = 10) of each drug.

$$\text{Bias} = (a+bX_c) - X_c$$

$$\%\text{Bias} = \frac{\text{Bias}}{X_C} \times 100 \quad (1)$$

a = y-intercept

b = slope

X_c = Critical value

$$\%\text{CV} = \frac{\text{SD}}{\text{Mean}} \times 100$$

$$CV_t = \sqrt{(CV_{\text{within-day}})^2 + (CV_{\text{between-day}})^2} \quad (2)$$

SD = standard deviation

CV_t = CV total

$$TE_{cal} = \%\text{Bias} + 3(CV_t) \quad (3)$$

$$TE_a = {}^1\!/_4 \text{ (normal range /mean of the normal range)} \quad (4)$$

RESULTS

Method precision

For determination of within-day (20 replicates in one day) and between-day (results from 20 consecutive days) precision, 3 levels of PreciControl ISD and PreciControl EVL (Roche Diagnostics) were tested. The results of within-day and between-day precision of the ECLIA assay are shown in **Table 1.** For CsA, the within-day precision ranged from 1.51% to 2.49%, and the between-day precision ranged from 3.07% to 5.05%. For EVL, the within-day and between-day precision ranged from 2.67% to 4.15% and 3.08% to 5.00%, respectively.

Table 1. Precision of ECLIA cyclosporine assay and ECLIA everolimus assay on the cobas e411 analyzer

Analyte	Within-day (n=20)			Between-day (n=20)		
	Mean (ng/mL)	SD (ng/mL)	CV (%)	Mean (ng/mL)	SD (ng/mL)	CV (%)
Cyclosporine						
PreciControl ISD level 1	91.98	2.18	2.37	86.11	4.35	5.05
PreciControl ISD level 2	335.62	5.07	1.51	313.59	14.82	4.73
PreciControl ISD level 3	1114.40	27.80	2.49	1092.15	33.56	3.07
Everolimus						
PreciControl EVL level 1	2.81	0.12	4.15	2.49	0.12	5.00
PreciControl EVL level 2	9.95	0.27	2.67	9.04	0.34	3.75
PreciControl EVL level 3	16.16	0.56	3.47	16.38	0.50	3.08

Abbreviation: ECLIA, electrochemiluminescence immunoassay; SD, standard deviation; CV, coefficient of variation; ISD level, immunosuppressant drug level; EVL level, everolimus level

Method comparison

Each individual blood sample was analyzed on both an Indiko Plus analyzer and a cobas e411 analyzer. The mean concentration ± standard deviation of 20 and 40 samples was similar between analyzers. Correlation equations, percentage of bias, and group mean bias between analyzers are shown in **Table 2.** Statistically significant correlations were determined by Pearson's correlation coefficient ($p<0.01$), and statistically significant differences were observed between group means, as determined by ANOVA ($p<0.01$). The calculated total errors were within acceptable range for both CsA and EVL (**Table 3**). Good linear correlations between the two analyzers are shown in **Figure 1-4.**

Table 2. Linear regression equations, Pearson's correlation coefficient ,percentage of bias, group means, and the differences among group means between the cobas e411 (Y) and the Indiko Plus (X) automated analyzers

Analyte	Regression equation	Pearson's, r *(p-value)*	r^2	bias (%)	X (mean±SD)	Y (mean±SD)	ANOVA *(p-value)*
Cyclosporine (n=20)	Y=-10.045+1.049X	0.9870 (<0.01)	0.9740	2.69	219.02±194.05	218.76±206.89	<0.01
Cyclosporine (n=40)	Y=-2.474+1.026X	0.9850 (<0.01)	0.9702	2.11	188.88±153.69	191.29±160.08	<0.01
Everolimus (n=20)	Y=-1.277+1.208X	0.9500 (<0.01)	0.9025	12.29	8.30±3.76	8.76±4.78	<0.01
Everolimus (n=40)	Y=-0.643+1.169X	0.9620 (<0.01)	0.9254	12.61	6.82±3.33	7.33±4.05	<0.01

Abbreviation: SD, standard deviation

Table 3. Therapeutic range, critical value, and allowable total error

Analyte	Therapeutic range (ng/mL)	Critical medical decision concentration (ng/mL)	Total allowable error (TE_a)	TE_{cal} (n=20)	TE_{cal} (n=40)
Cyclosporine	100-400	500	±30%	17.03	16.45
Everolimus	3-8	15	±30%	27.95	28.27

Abbreviations: TE_a, total error; TE, total error; TE_{cal}, calculated total error

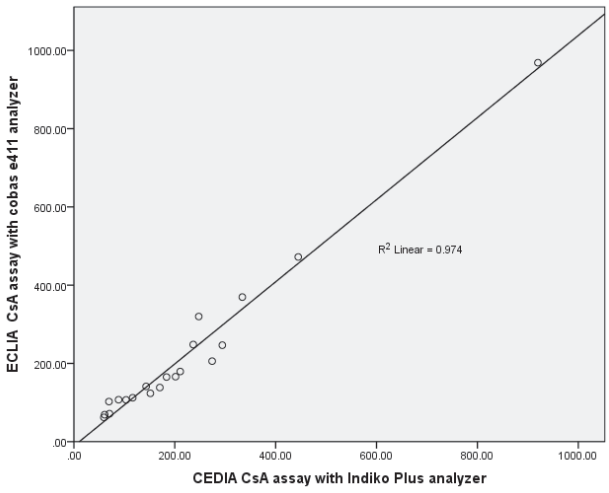

Figure 1. Linear correlation between cyclosporine concentrations determined by ECLIA on the cobas e411 and values determined by CEDIA on the Indiko Plus in 20 patient samples

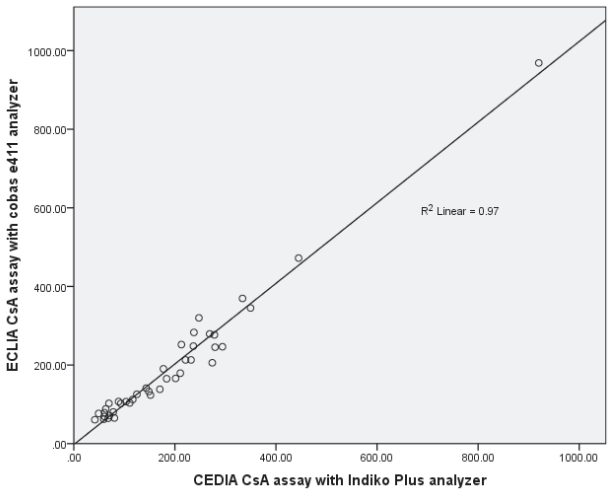

Figure 2. Linear correlation between cyclosporine concentrations determined by ECLIA on the cobas e411 and values determined by CEDIA on the Indiko Plus in 40 patient samples

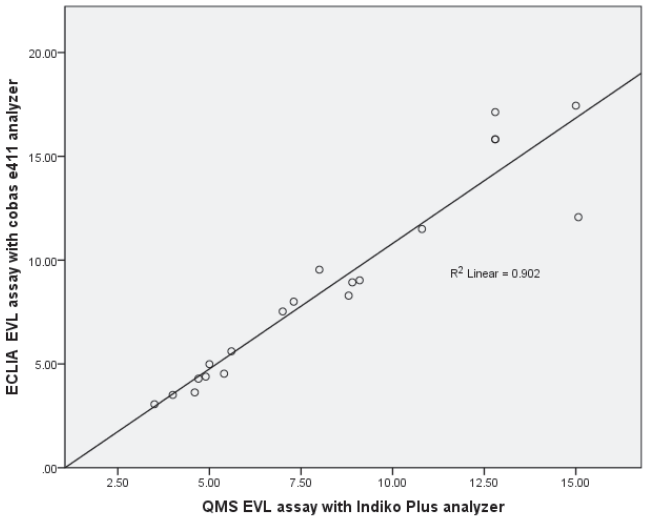

Figure 3. Linear correlation between everolimus concentrations determined by ECLIA on the cobas e411 and the values determined by QMS on the Indiko Plus in 20 patient samples

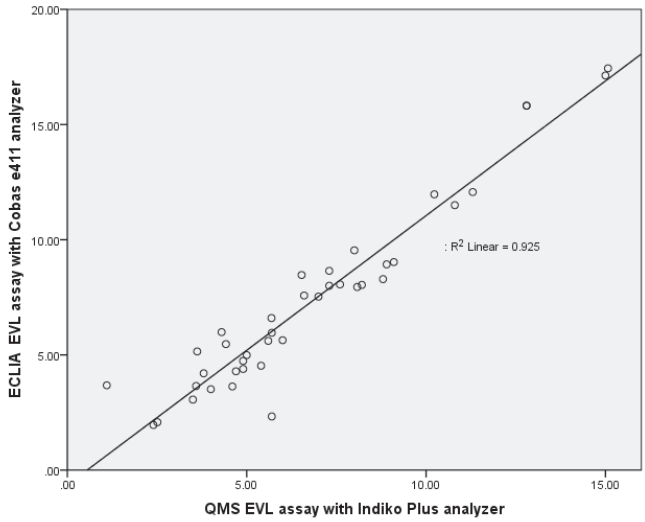

Figure 4. Linear correlation between everolimus concentrations determined by ECLIA on the cobas e411 and the values determined by QMS on the Indiko Plus in 40 patient samples

DISCUSSION

In this study, we evaluated the analytical performance of the ECLIA CsA and ECLIA EVL assays. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been recognized as a gold standard method for the accurate analysis of CsA and EVL concentrations in therapeutic monitoring. However, automated immunoassay methods become an important role in a modern routine clinical laboratory and replace commonly used. Several previous comparative studies reported differences between LC-MS/MS and other blood level measurement methods, but the results of all evaluated methods were acceptably well-correlated with the results of LC-MS/MS.[6-8, 19-24]

The ECLIA EVL and QMS EVL immunoassays both correlated well with LC-MS/MS.[6, 19-21] One study reported a 34.2% group mean bias between LC-MS/MS and the ECLIA EVL assay, with a small but significant -8.0% bias reported between LC-MS/MS and the QMS EVL assay.[21] Moreover, the EVL concentrations determined by ECLIA EVL assay were consistently systematically higher than those measured by QMS EVL assay.[6, 19-21] In the present study, we found similar results between the two assays. The ECLIA EVL assay was well-correlated with the QMS EVL assay, but ECLIA EVL showed higher concentrations than QMS EVL [5.74% (n=20), and 7.51% (n=40) group mean biases].

The ECLIA CsA and CEDIA CsA immunoassays both correlated well with LC-MS/MS.[7, 8, 22-24] CsA concentration by ECLIA was higher than the result by CEDIA, but both demonstrated a mean difference compared to LC-MS/MS.[7] In the present study, we found comparable results between the ECLIA and CEDIA CsA assays (r=0.9740 for n=20, and r=0.9702 for n=40). However, the ECLIA CsA assay showed higher concentrations than the CEDIA CsA assay when n=40 (1.28% group mean bias), but slightly lower concentrations than

the CEDIA CsA assay when n=20 (-0.12% group mean bias).[21, 25]

The ECLIA CsA and ECLIA EVL assays showed good precision with a reasonable LOQ, good linearity, and good correlation with the CEDIA and QMS assays. There were statistically significant differences between group means as determined by ANOVA. Immunoassays are usually suffering from cross-reactivity, significantly from metabolites of the parent drug, which will result in overestimation of drug concentrations.[25] In this study, both drugs measured results by cobas e411 were higher than those from the Indiko plus is probably caused by cross reactivity with metabolites like the previously study.[21] The calculated total error of EVL is higher than the measurement of CsA can be caused by a higher bias between two assays. However, the calculated total error was within acceptable range for both CsA and EVL at both n=20 and n=40 (<30%). Thus, the ECLIA CsA and ECLIA EVL assays are suitable for routine therapeutic monitoring. In this study, differences in sample size did not affect the results of comparison.

CONCLUSIONS

The results of this study revealed that ECLIA on the cobas e411 system demonstrated good analytical performance that correlated well with the results of the CEDIA and QMS assays on the Indiko Plus system. Accordingly, the cobas e411 system should be introduced into routine practice for determination of cyclosporine and everolimus blood levels.

CONFLICT OF INTEREST DECLARATION

All authors declare no personal or professional conflicts of interest, and no financial support from the companies that produce and/or distribute the drugs, devices, or materials described in this report.

FUNDING DISCLOSURE

This study was supported by a grant from the Siriraj Research Development Fund [managed by Routine to Research (R2R)], Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (grant no. R016315006).

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