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Application of Electric Power Purification Instrument to Fluorescence Polarization Immunoassay for Cyclosporine A Determination

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Effects of the electric power purification instrument (EPPI) on the precision of cyclosporine A (Cs A) assay were tested by using fluorescence polarization immunoassay (FPIA).The power supply environment in a room for drug measurement was evaluated using a digital multi-meter and an oscilloscope. Sags (short drops in voltage), surges (sudden, momentary rises in voltage) and wide fluctuations (92.2 − 101 V) in voltage during the day were discovered. Although no change of intra-day (within-run assay) coefficient of variations (CVs) and assay ranges were observed with attachment of the EPPI, inter-day CVs (between-run) in concentration (100 ng/mL) of Cs A were significantly improved. The average of CVs was 2.0% (CV range: 1.21 to 3.19%) with the EPPI and 3.3 % (CV range: 2.1 to 4.2%) without it. Inter-day CVs for Cs A concentration (25 ng/mL) with attachment of the instrument was slightly more suppressed (CV range: 2.4 to 8.1%) than without it (CV range: 3.2 to 8.7%). It is suggested that stabilization of the power supply voltage of a TDx system is useful for improving inter-day variation of Cs A determination.

KeyWords : Fluorescence polarization immunoassay, cyclosporine, power supply, precision, electric power purification instrument, TDx system

INTRODUCTION

Cyclosporine A (Cs A) has been used for treatment of rejection of kidney, liver, and bone marrow transplants; suppression of graft versus host disease; Behcet's disease, psoriasis, aplastic anemia, and nephrosis syndrome. Cs A has also been used on a trial basis to treat neurological disorders[1-5)] and autoimmune hepatic disorders such as primary biliary cirrhosis[6-9)]. It has been effective in some patients with such disorders with therapeutic concentrations of 150 ng/mL and lower[5-7)]. This implies that ensuring the reliability of low-concentration Cs A assays will become more important in a clinical setting. Fluorescence polarization immunoassay (FPIA) is used in many medical institutions for monitoring a variety of drug concentrations in blood because of its convenience. A TDx system is guaranteed to operate at a 100 volts, which is the voltage ordinarily used in Japan. However, when a multitude of instruments are used in hospitals, their effect on the power supply environment is significant. This study was conducted to investigate the effects of changes in voltage on blood concentration measurement and examine to what extent stabilization of voltage affects the assay precision of a TDx system using Cs A as the assay drug.

MATERIALS AND METHODS

Apparatus

A TDx system (Abbott Co. Ltd., Abbott Park North Chicago, IL, USA) was used for FPIA. An electric power purification instrument (DI-H102)

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Sample Preparation and Measurement of Cs A Concentrations

Twenty-five ng/mL of Cs A concentration was prepared by diluting Calibrator B (100 ng/mL) with Calibrator A (0 ng/mL), and Calibrator B (100 ng/mL) and Control L (150ng/mL) was used in this study, and the Calibrators and the Controls were purchased from Abbott Co. Ltd.. After advanced preparation of samples based on established methods, supernatant was pooled by concentration and refrigerated until it was used. Measurement of Cs A was fully automated. A TDx system was used with Cyclosporine-SP Dynapak (Abbott Co. Ltd., IL, USA) from the same lot and calibration was performed only once.

Power supply environment in a room of drug measurement

The power supply environment of a measurement instrument equipped with a TDx system in a room was investigated using a digital multi-meter (R6551, Advantest Corporation, Tokyo, Japan) and an oscilloscope (TDS460, Tektronix Inc., Oregon, USA).

Intra-day CV% and range

Control L (150ng/mL, Abbott Co. Ltd.) was divided into 19 cuvettes and measured 4 times a day (10:00 am, 1:00 pm, 4:00 pm and 7:00 pm) by TDx systems. A range was calculated from the difference between maximum, CV% was calculated from these 4 assays.

Inter-day CV%, bias %, and check of photo energy

Samples (n = 3) of Cs A with a concentration of 25 ng/mL and 100 ng/mL were measured once a day over 6 days by the EPPI attached input unit of the TDx system.

The CV % was determined from dividing SD by the average. The mean bias % was obtained from dividing the mean measurement value of Cs A concentrations (n = 3) in each day by the average of measurements for six days. A photo check was performed at 9:00 am once a day for 5 days according to an attached the TDx manual. Standard carousel's gain, average intensity and average polarization was 5, 10600 and 47.5 mp, respectively.

Statistics

Statistical analysis was performed using non-parametric analytic procedure, (Mann-Whitney U-test) in the Stat View program version 5.0 (SAS Institute Inc., Cary, NC, USA), and p value of <0.05 was considered to be statistically significant.

RESULTS

There is a cable joint chart TDx system without the EPPI and with the EPPI as shown in Fig.1A and Fig1B, respectively. The results of measurements with a digital multi-meter and an oscilloscope in the laboratory without EPPI are shown in Fig. 2.

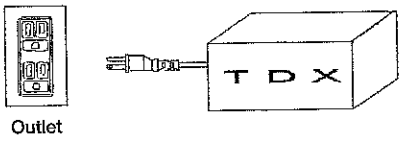


Fig. 1A. Conventional cable joint of TDx system in a TDM room.

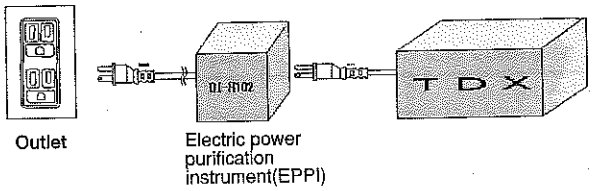


Fig.1B. Connection between TDx system and electric power purification instrument (DI-H102).

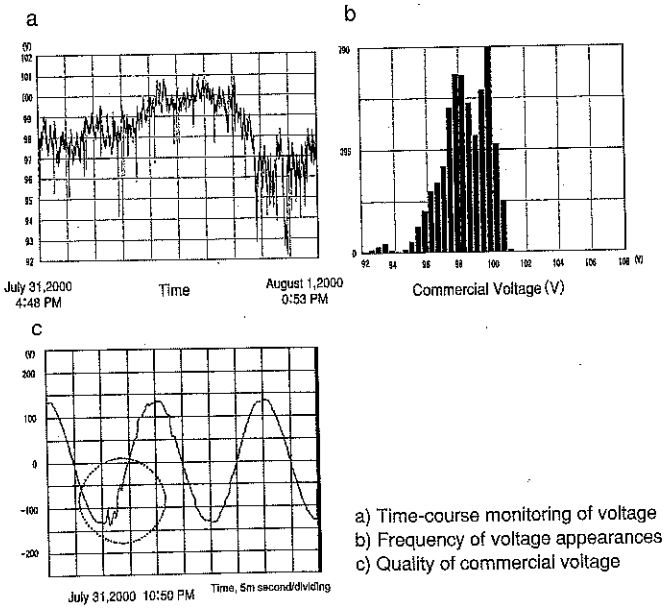
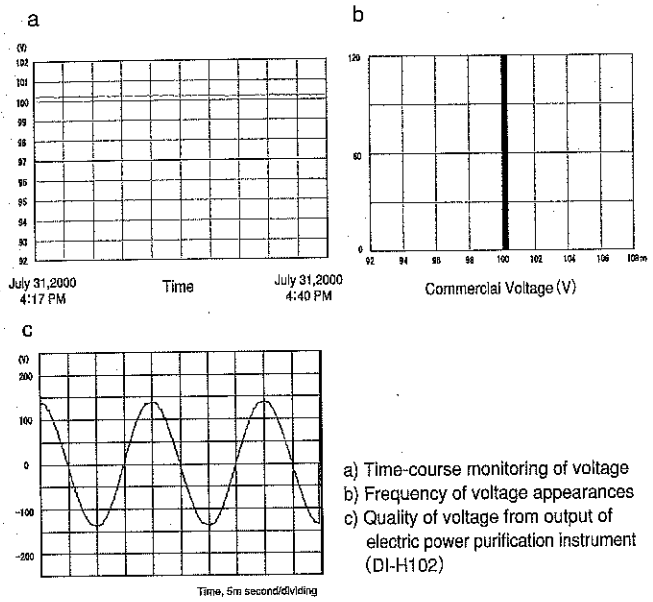


Fig. 2. Commercial voltage monitoring in our laboratory.

The voltage range at the power supply input unit of the TDx system without the EPPI, was 92.2V−101.1V, (variation of 8.9V), and the median

was 98v. Thus, the drug measurement room was suffering from a chronic lack of voltage. In addition, large fluctuations in voltage were noted. Detection of surges, sags, and impulses was confirmed as shown in Fig 2. However, when the EPPI was attached, the voltage was very stable with a median of around 100V, neither voltage variations nor shortages were noted (Fig. 3), and neither surges nor sags were detected.



a) Time-course monitoring of voltage
b) Frequency of voltage appearances
c) Quality of voltage from output of electric power purification instrument (DI-H102)

Fig. 3. Voltage monitoring of input portion of the TDx system after attachment of electric power purification instrument (DI-H102).

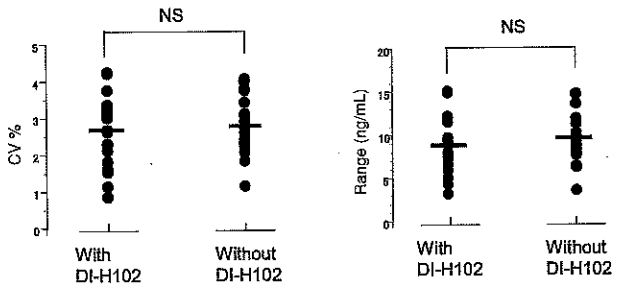


Fig. 4. Effect of electric power purification instrument (DI-H102) on intra-day CV % and range in TDx system for Cs A assay. The bar represents mean of 19 experiments.

Effect of electric power purification instrument (DI-H102) on intra-day CV % and range in TDx system for Cs A assay as shown in Fig 4. This study made comparison by CV% how much difference there was in the concentration measurement of cyclosporine A between TDx system attached with and TDx system without EPPI in a concentration of 100 ng/mL. There was no change in CVs and the range of recordings with and without the EPPI. Width of observation of bias % with the EPPI in Cs A (100 ng/mL) is slightly small relative to the diagram without the EPPI, as shown in Fig. 5.

Table 1. Effect of DI-H102 on inter - day variation in cyclosporine A measurement.

	With DI-H102	Without DI-H102	P value
CV (%)	2.03 ± 0.79	3.25 ± 0.90	0.0250
Bias (%)	100 ± 2	100 ± 3	NS
Rande (ng/ml)	3.85 ± 1.43	6.24 ± 1.70	0.0250

The assay (n=3) was performed once a day for 6 days and tested the concentration of whole blood cyclosporine A (100 ng/ml). The value represents mean ± SD. NS: No significant

Table 2. Effect of DI-H102 on inter- day variation in cyclosporine A measurement by using low concentration.

	With DI-H102	Without DI-H102	P value
CV (%)	5.27 ± 1.91	6.37 ± 2.07	NS (0.3367)
Bias (%)	99.8 ± 3.98	100 ± 6.33	NS (0.7488)
Rande (ng/ml)	3.28 ± 1.24	4.06 ± 1.41	NS (0.2623)

The assay (n=3) was performed once a day for 6 days and tested the concentration of whole blood cyclosporine A (25 ng/ml). The value represents mean ± SD. NS: No significant

These results indicated no effect on intra-day variation of cyclosporine A. Inter-day CVs of Cs A (100 ng/mL) are shown in Fig. 6, and the average of CVs without the EPPI was 3.3 % (n=6), and suppressed to 2.0 % while using it. Inter-day CVs and range due to using the EPPI resulted in a statistically significant ($p<0.05$), as shown in Table1. When using 25 ng/mL of Cs A (the detection limit of a TDx system), none of the reducing effects of the EPPI on CV % were statistically significant, however there was a tendency to reduce the variations with the EPPI (Fig. 6).

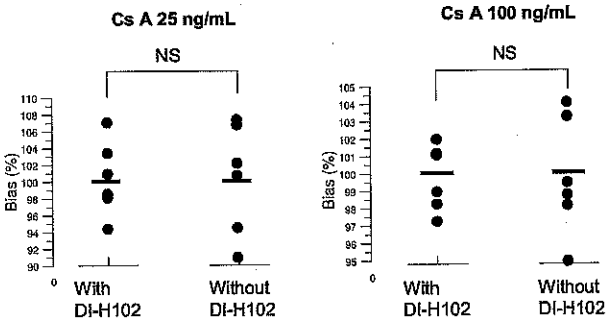


Fig. 5. Effect of electric power purification instrument (DI-H102) on inter-day bias in TDx system for Cs A assay. The bar represents mean of the points for six days. Each point was calculated from triplicate measurements in a day.

In order to evaluate inter-day variation of optical energy in the TDx system, a photo check was performed with the EPPI attached for 6 days (Monday to Saturday). There were no effects on average intensity, intensity range, and average polarization or polarization range by the EPPI, while standard deviation of average intensity with attachment of the EPPI was pressed to 31.7% of that without it, as shown in Table 3.

Table 3. Effect of electric power purification instrument (DI-H102) on photo energy in TDx system

	With DI-H102	Without DI-H102
Average intensity	10058 ± 20	10071 ± 63
Intensity range	346 ± 16	357 ± 20
Average polarization (mp)	47.5 ± 0.1	47.2 ± 0.4
Polarization range (mp)	1.56 ± 0.34	1.53 ± 0.35

There is no unit of average intensity because it is amplified by photo multiple tube (PMT). The value represents mean ± SD of 5 experiments.

DISCUSSION

Advances in medicine have resulted in the introduction of a variety of instruments into hospitals. These instruments consume a considerable amount of electric power. The standard service voltage in Japan is 100V, but the voltage is often lower when it is raining and during the summer when power consumption is high. The TDx system is designed for the United States specification (115V) and is guaranteed to operate with voltages of 100±10 in Japan, However, medical institutions cannot always maintain a stable voltage of near 100V. It was confirmed in our hospital by voltage monitoring that the voltage drops below 95V over several hours. At present, the extent to which the power supply environment affects the TDx system for Cs A assay is unknown, and it is not known whether the TDx assay might be affected by voltage fluctuations. Meanwhile, Cs A is measured by the TDx system in our institution, and 38 % of all such measurements involve concentrations of 100 ng/mL or less. Consequently, evaluation of measurement precision at voltage fluctuations is important.

The means of intra-day CVs was 3.3 % in 100 ng/mL of the concentration without the EPPI. This is smaller than that of the report (CV=9.7 % in 136 ng/mL, n=3) by the other study[10] or the description (CV=3.9% in 146.7ng/mL, n=550) in an assay kit calligraphy[11]. This discrepancy may be attributed to use of the pooled sample to remove handling error in the extract effect. Attachment of the EPPI for improving the power supply environment resulted in stable voltage and little noise, and good quality current was imputed to the TDx system (Fig. 2). The effect of the EPPI was not significant in intra-day CVs. The amount of voltage fluctuation in the hospital depends on the day of the week and the weather, and therefore the EPPI is more effective against inter-day variations than intra-day (Fig. 6). The stability of optimal intensity may contribute slightly to precision in measurement; however, a more significant factor could be the stability of temperature inside the TDx system. When the voltage drops, optimal air circulation is disrupted and it leads to variances in temperature within the TDx system.

Twenty-five to twenty-six ng/mL of Cs A concentration is the lower detection limit for the TDx system[12, 13, 14] and this condition might involve relatively high background noise. Therefore, this concentration may be too low to establish the effect of the EPPI.

The photo check was in the latitude based on the TDx system standard carousel regardless of whether or not the EPPI was present. Average intensity and standard deviation increased slightly when the EPPI was not used (Table 3). According to the announcement of Abbott Co. Ltd., TDx is guaranteed for its proper operation with voltage more than 95V. Like almost home electric appliances, this can operate even below the specified external voltage to some extent because the transformer controls changes of the internal voltage against changes of the external voltage. In fact, we checked whether TDx operates by decreasing supplied voltage to 85V and it did not work but worked with 90V. When the voltage decreases, average intensity from photo multi tube in the TDx system might be amplified by the regulatory system, and it is easy for the noise to be amplified at the same time. The EPPI observed no polarization change based on standard carousel. Pipetting and temperature checks were examined to explore the effect of attachment of the EPPI on

the TDx system; however, none of the checks revealed any differences when the EPPI was attached (data not shown).

In the treatment of autoimmune disorders such as Behcet's disease and nephrosis syndrome, it is desirable to adjust the trough concentration of Cs A to 50−200 ng/mL and 100 ng/mL or less, respectively[15]. In the maintenance therapy phase of transplant patients, it is recommended that the concentration of Cs A in the blood is adjusted to a trough level of 80−120 ng/mL[15]. Experimental Cs A treatment has been attempted in refractory autoimmune disorders, though target trough concentrations are often not clear. Trough concentrations for many neurological disorders are set even lower than those for prevention and treatment of graft rejection. Therefore, the accuracy and the precision managements of lower concentration of Cs A than Control L (150 ng/mL), and a stable voltage and good quality power supply are important. Though the decrease of CV% is significant statistically in cyclosporine A quantitation of TDx system attached with EPPI, it is unlikely at this point that the attachment of EPPI would affect the medication plan by doctors because its improved value is small. However, we think it is sure that the implementation of more accurate TDM would strengthen trust between the department of TDM or analytical staff and doctors. In addition, EPPI has battery inside and it can be used as an emergency power source for TDM room.

In conclusion, there are large variations in voltage and tin pot current in the therapeutic drug monitoring laboratory in our hospital. This study highlights the importance of identifying the power supply environment in rooms where blood concentrations are measured, and suggests that a stable voltage supply and a quality power supply to the power input unit of a TDx system are possible by use of the electric power purification instrument (DI-H102) because of the possible suppression of an inter-day variation.

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