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Therapeutic drug monitoring of tacrolimus with the dried blood spot method

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Abstract

In a preliminary investigation an assay for tacrolimus based on fingerprick sampling and consecutive application as a blood spot on sampling paper has been developed. The dried blood spot was analysed by HPLC–tandem mass spectrometry. The validated range was 1–30 μg/l. Intra- and inter-assay variability for precision and accuracy was <7.5% and 15%, respectively. Tacrolimus concentrations of 24 stable out patients were compared after both blood spot sampling and conventional venous sampling. Method agreement was investigated with the methods of Passing and Bablok and Bland Altman and proved suitable for clinical use. The dried blood spot method for tacrolimus seems promising for patient monitoring.

Keywords: Tacrolimus; Dried blood spot; Fingerprick; Tandem mass spectrometry; Therapeutic drug monitoring

1. Introduction

Lifetime periodic therapeutic drug monitoring (TDM) of the immunosuppressive drug tacrolimus (see Fig. 1) in transplantation patients is necessary, because of a narrow therapeutic window and large inter- and intraindividual variability of the tacrolimus blood concentrations [1]. The assay of tacrolimus is usually performed in EDTA blood, obtained by venous sampling [1]. Dried blood spot sampling (DBS) could be an alternative. DBS capillary blood is obtained from a fingerprick with an automatic lancet by the patients themselves. Consecutively the drop of blood is applied to sampling paper. After drying, the paper with the blood spot sample is sent by mail to the laboratory. The laboratory punches out a disk from the blood spot. The disk is extracted. The filter paper disk provides a volumetric measurement that is similar to liquid measurement devices. Possible advantages of therapeutic drug monitoring with DBS are: (1) The patients do not have to leave home and no phlebotomist is necessary. (2) Monitoring results are known, when patients visit the clinician for routine control. (3) Monitoring at any desired sampling time can conveniently be done and a shortened Area Under the blood Concentration time Curve (AUC) can be constructed.

Dried blood spot sampling has become common practice for newborns. Over the past decade many applications have been reported for both qualitative and quantitative screening of metabolic disorders [2].

In literature assays based on the dried blood spot method have been reported for several drugs such as antimalarials, antiepileptics, antiretrovirals, metformin and paracetamol [3–7]. In the Epilepsy Institute of The Netherlands in Hoofddorp the dried blood spot method is successfully used in daily practice for many years now [8]. Usefulness of capillary blood obtained by fingerprick for TDM of tacrolimus has been described recently [9]. However, a method for dried blood spot sampling of tacrolimus has not yet been reported in literature.

In this article we present a new method for monitoring tacrolimus concentrations in blood by sampling with the dried

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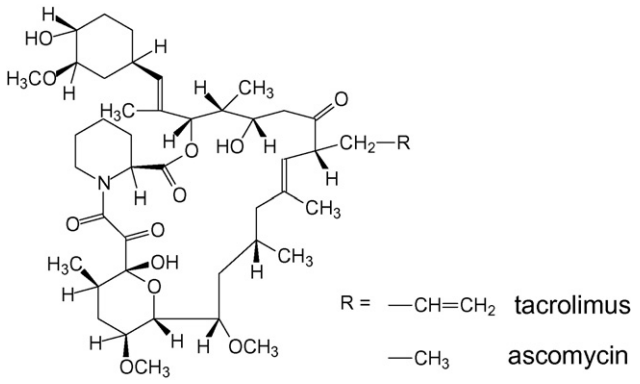

Fig. 1. structures of tacrolimus and the internal standard ascomycin.

blood spot technique followed by analysis with HPLC–tandem mass spectrometry. The developed dried blood spot method was tested for clinical use. Patient tacrolimus concentrations in blood spot samples and venous blood samples, drawn at the same time, were compared.

2. Experimental

2.1. Routine assay for tacrolimus in venous blood

The routine tacrolimus assay in venous blood in our laboratory is based on HPLC–tandem mass spectrometry. The assay method for tacrolimus in venous blood samples and our newly developed assay in blood spot samples are essentially the same with exception of the sample preparation procedure (see Section 2.6.1).

The routine assay in venous blood is linear from 1 to 300 μg/l. Intra-assay precision and accuracy was 3.4%, 2.2%, 3.0% and 102%, 94% and 94%, respectively at 3.04, 6.23 and 13.0 μg/l ($n = 6$). Inter-assay precision and accuracy was 8.2%, 5.2%, 4.6% and 102%, 94%, and 93% ($n = 9$), respectively. Limit of quantification was 1.0 μg/l. The results of our quality control (6.7 μg/l) were 98.5% of the nominal value ± 6.2% ($n = 300$). Our laboratory also participates in the international tacrolimus proficiency testing scheme. Results of the last 15 months showed satisfactory accuracy: 99.3 ± 6.1% of the method mean ($n = 45$) [10].

2.2. Chemicals

Tacrolimus was a gift obtained from Fujisawa (München, Germany).

The internal standard (IS), ascomycin (see Fig. 1) was obtained from Biosciences (Darmstadt, Germany).

Methanol, ethanol, and acetonitrile (all LiChrosolv), formic acid 98–100% (Suprapur), ammonium acetate and zinc sulphate heptahydrate (all Pro Analysi) were obtained from Merck (Darmstadt, Germany).

ClinChek®-controls for immunosuppressants no 8833, lot no. 413 (Recipe Chemicals and Instruments, Munich, Germany) were used.

Sampling paper: filter paper, item no. 10535097, was obtained from Whatman, Schleicher and Schuell (Dassel, Germany).

Peelable Heat Seeling Foil Sheets were from Westburg (Epsom, UK).

Tacrolimus-free venous blood was obtained from surplus patient EDTA-blood for routine therapeutic drug monitoring.

CPD (citrate phosphate dextrose) donor blood was obtained from Sanquin blood bank (Nijmegen, The Netherlands).

2.2.1. Reagents

2.2.1.1. Standard solutions. Stock solutions of tacrolimus (1000 mg/l) and ascomycin (1000 mg/l) were prepared in ethanol. Ascomycin stock solution was used to prepare an internal standard (IS) working solution (10 mg/l) in ethanol. Tacrolimus stock solution was used to prepare a tacrolimus working solution (10 mg/l) in ethanol. All solutions were stored at darkness at +4 °C. The tacrolimus working solution and the ascomycin working solution proved stable for at least 12 months.

2.2.2. Eluent A

Two millimolars of ammonium acetate in water + 0.1% (v/v) formic acid (0.31 g ammonium acetate is dissolved in 2000 ml water and 2 ml formic acid is added).

2.2.3. Eluent B

Two millimolars of ammonium acetate in methanol + 0.1% (v/v) formic acid (0.31 g ammonium acetate is dissolved in 2000 ml methanol and 2 ml formic acid is added).

2.2.4. Internal standard solution for venous blood analysis

Ten microlitres of the internal standard working solution in ethanol (10 mg/ml) is diluted with methanol to 25 ml.

2.2.5. Precipitation reagent with internal standard

Precipitation reagent is used for venous blood samples and is freshly prepared daily. The internal standard solution is mixed with a solution of zinc sulphate heptahydrate 89 g/l in water in a 4:1 (v/v) ratio.

2.2.6. Extraction solution for blood spot analysis with internal standard

Twenty five microlitres of IS working solution (10 mg/l) is diluted to 25.0 ml with methanol. This solution is 10 times further diluted with methanol:acetonitrile 40:10 (v/v).

2.3. Instrumentation and chromatographic conditions

2.3.1. On line extraction and chromatographic conditions

Chromatography was performed using a Waters 2795 Alliance HPLC system (Waters Ltd., Watford, UK). After injection the prepared sample was analyzed by on line solid phase extraction using a Waters Oasis HLB cartridge column, (2.1 mm × 20 mm, 25 μm) cat. no. 186000706. After flushing 0.5 min with solvent A:solvent B = 40:60 (v/v) at a flow rate of 3 ml/min, the analytes were foreflushed to a Waters Atlantis

dC18 (3.0× 100 mm, 5 μm) column, with solvent A:solvent B = 3:97 (v/v) at a flow rate of 0.75 ml/min. Four minutes after start of the cycle the flow rate was set on 1.5 ml/min. After 6 min the column switching device was switched to the starting position to equilibrate for the next injection 30 s later. The analytical column was maintained at 60 °C. The cycle time was 6.5 min from injection to injection.

2.3.2. Mass spectrometry

A Quattro Micro tandem mass spectrometer fitted with a Z spray ion source was used (Micromass, Manchester, UK). The instrument was operated in the electrospray-positive ionization mode and was directly coupled to the HPLC system. System control and data acquisition were performed with MassLynx 4.0 software.

A solution of tacrolimus or ascomycin (1 mg/l) in mobile phase was infused into the ion source, and the cone voltage was optimized to maximize the intensity of the $(M + NH_4)^+$ precursor ions of tacrolimus and ascomycin (*m/z* 821.4 and 809.4, respectively). The collision energy was then adjusted to optimize the signal for the most intense product ions (*m/z* 768.3 and 756.3, respectively). Typical tuning conditions were as follows: electrospray capillary voltage, 1.0 kV; sample cone voltage, 24 V; and collision energy, 19 eV.

Source temperature, 120 °C; desolvation temperature: 350 °C. Desolvation gas (nitrogen) flow: 811 l/h. Collision gas was argon.

Sample analysis was performed in the multiple reaction monitoring mode of the mass spectrometer with a dwell time of 0.2 s per transition. Calibration curves were constructed using linear least square regression with 1/*x* weighting.

2.3.3. Hole puncher

Paper disks with a diameter of 7.5 mm were punched with an electromagnetic driven hole puncher, especially developed for blood spot analysis of drugs by P. Edelbroek (SEIN, Hoofddorp, The Netherlands) in cooperation with the department of Mechatronics Engineering, University Medical Centre, Leiden, The Netherlands.

2.3.4. Seal apparatus

The Eppendorf cups were sealed with Peelable Heat Sealing Foil sheets by a Westburg sealing apparatus.

2.4. Sampling

Venous sampling was done by venapuncture and the samples were collected in vacutainer tubes (Becton and Dickinson, Franklin Lakes, NJ, USA) containing EDTA and stored at 4 °C until analysis.

Fingerprick blood samples were collected using Glucolet 2 Automatic Lancing Device (Bayer, Mishawaka, USA). Samples were collected from the fingertip. The first drop was discarded and the next drop collected to fill a 8 mm premarked circle on the above mentioned sampling paper. The blood spots were allowed to dry at room temperature overnight.

2.5. Preparation of calibration standards and quality control samples

2.5.1. Calibrations standards in blood for blood spot method

Tacrolimus working solution (10 mg/l) was diluted with water to a concentration of 500 μg/l. This solution is added to fresh tacrolimus-free EDTA blood to obtain a 50 μg/l solution of tacrolimus in blood. After further dilution with fresh (tacrolimus free) EDTA blood, calibration standards were obtained: 1, 2, 3, 6, 9, 15 and 30 μg/l. Also a blank standard was included.

2.5.2. Quality control standards

Lyophilized ClinChek® quality control standards were reconstituted with water as described in the insert leaflet and stored at −20 °C.

Also quality controls were prepared in our laboratory. Three levels of quality control blood pools (4, 10 and 24 μg/l) were prepared in the same way as the calibration standards with fresh tacrolimus free EDTA-blood and were stored at 4 °C. The quality control standards and the calibration standards were compounded separately as different batches.

2.5.3. Calibration standards and quality controls on sampling paper

Thirty microlitres of each calibration standard or quality control were applied onto sampling paper and kept overnight at room temperature to dry.

2.6. Sample preparation

2.6.1. Sample preparation of venous blood

In an Eppendorf cup 200 μl blood was mixed with 200 μl precipitation reagent (see Section 2.2.4) on a vortex mixer until homogenous. After 10 min waiting, the cups were mixed again. Then the cups were centrifuged at 10,900 rpm for 5 min at room temperature. The supernatant was transferred to another Eppendorf cup and 20 μl was injected into the HPLC system.

2.6.2. Sample preparation of the dried blood spots

Paper disks were punched out of the dried blood spots on the filter paper and placed into glass tubes.

Twohundred and fifty microlitres of extraction solution were added. The tubes were shaken on a mechanical shaker for 60 min. The liquid phase was pipetted to an Eppendorf cup. To avoid evaporation the cups were sealed with Peelable Heat Sealing Foil sheets. An aliquot of 50 μl of the samples was injected into the HPLC system.

2.7. Method development: preliminary studies

A preliminary study of the visual aspects and the weight of the paper disks with blood spots was performed. The weight of the blood spots was determined by weighing the paper disks with the blood spot. The mean weight of three blank paper

disks was substracted. Parameters influencing blood spots were investigated.

2.7.1. Type of blood

Thirty microlitres of CPD blood, fresh tacrolimus free EDTA blood and ClinChek® quality controls, respectively were applied to sampling paper and dried overnight. The weight of the punched out paper disks with blood spots were compared. The CPD blood used was hemolytic: after centrifugation not any separation of blood corpuscles and plasma could be observed. Before punching out the disks, the diameters of the spots on the sampling paper were also measured.

2.7.2. Variation of blood volume

Blood spots were made by application of 10, 20 and 30 μl fresh blood respectively to sampling paper and dried overnight. The weight of the punched out paper disks was investigated.

2.7.3. Variation of drying time

Thirty microlitres of fresh blood was applied to sampling paper and paper disks were punched out after different drying times (1, 4, 18 h = overnight drying). The weight of the paper disks was investigated.

2.8. Analytical method validation

Analytical method validation was conducted according to the Guidance for Industry [11].

2.8.1. Accuracy, precision, limit of detection and lower limit of quantification

The accuracy and precision of the method were determined by analysis of 30 μl spiked fresh venous blood onto the sampling paper at three different concentrations. Analysis was done on 6 days in duplicate during a period of 8 days: inter-assay. On the fourth day every concentration level was analysed six times: intra-assay. Concentrations were determined with freshly prepared calibration standards and intra- and inter-assay precision and accuracy were calculated. Acceptance criteria were: precision and accuracy <15%. The limit of detection was based on peak height 3× the largest baseline fluctuation in a 1 min window around the elution time of the analyte in an analytical blank. The lower limit of quantification was defined as the concentration where the analyte response of a spiked sample had a precision of ≤20% and accuracy of ±20% [11].

2.8.2. Linearity

Calibration graphs were constructed using 7 standards and a blank (2.5.1). The ratio of the tacrolimus peak area and the internal standard area at each concentration was plotted as a function of the tacrolimus concentration and $1/x$ weighted linear regression was performed. The calibration graph was forced through zero.

2.8.3. Extraction recovery

The tacrolimus peak areas from bloodspots of quality controls were compared with the areas after direct injection of the standards at the same nominal concentrations as after reconstitution. The paper spot was cut out with a wide margin in order to recover the complete blood spot.

2.8.4. Selectivity of blood spot method

In order to investigate if interfering endogenous compounds are present, surplus venous EDTA blood from tacrolimus-free routine samples of six different patients were analysed with the blood spot method. The blood spots were extracted without internal standard.

2.9. Stability of dried blood spots

The stability of the dried blood spot samples was tested with calibration standards, quality controls and patients. Our goals were: (1) investigating stability of dried blood spot samples of patients during simulated transport conditions, (2) investigation of stability of calibration standards and controls of dried blood spots in order to investigate if batchwise preparation and subsequent storage would be possible.

2.9.1. Patients

Several duplicate blood spots samples were collected. One of the blood spots samples was analysed immediately after overnight drying. The second blood spot sample was stored during several days at different temperature conditions (70 °C, 37 °C, room temperature, and at −20 °C).

2.9.2. 2.9.2 Calibration standards and quality controls

30 μl of each calibration standard or ClinChek® quality control is applied to the sampling paper. After drying for one night at room temperature the papers are stored at +4 °C. After 9, 13 and 31 days the blood spots were analysed and compared with freshly prepared blood spots with calibration standards.

2.10. Comparison of blood spot and venous samples of patients

Trough tacrolimus blood concentrations of kidney transplant patients are monitored routinely every 3 months. In the outpatient department both venous and blood spot samples were collected of 24 patients. The patients got a brief written and verbal instruction about the technique. It was emphasized to let flow a big drop of blood in the middle of the predrawn circle until the circle is completely filled and to allow sufficient drying time. On arrival in the laboratory the blood spots were visually inspected. Criteria were: complete, homogenous and symmetric filling of the 8 mm circle and dark red even colour on both sides of the paper.

Assays were performed the next day. The study was approved by the local medical ethical board.

2.11. Statistics

The nonparametric regression procedure of Passing and Bablok was used for method comparison [12]. Agreement

Table 1
Validation: precision and accuracy intra-and interassay

	ClinChek® (6.60 μg/l)	Control (4.0 μg/l)	Control (10.0 μg/l)	Control (24.0 μg/l)
Inter-assay (*n*)	21	6	6	6
Mean (μg/l)	4.92	3.67	8.77	21.13
S.D. (μg/l)	0.27	0.27	0.45	1.15
CV (%)	5.4	7.4	5.1	5.4
% found of tacrolimus added	75	92	88	88
Intra-assay (*n*)	6	6	6	6
Mean (μg/l)	5.08	3.67	8.70	20.61
S.D. (μg/l)	0.32	0.22	0.53	1.54
CV (%)	6.3	6.0	6.0	7.5
% found of tacrolimus added	77	92	87	86

between methods was presented using the Bland Altman difference plot [13,14].

3. Results

3.1. Method development: preliminary studies

3.1.1. Influence of different type of blood on blood spot weight

Lower weight was found for dried blood spots made with CPD blood and ClinChek® quality control standards in comparison with fresh blood: 3.24 mg ± 3.6%, 2.59 mg ± 4.5%, 3.56 mg ± 4.0%, respectively ($n = 6$). With fresh blood the punches looked evenly dark red coloured at both sides. With the other blood types the punches were not evenly coloured at both sides and the colour was more intense at the application site. Moreover the diameter of the spots on the sampling paper of each of the blood types was different: CPD blood 10.2 mm ± 3.3%, Clinchek® 11.0 mm ± 0.6% and fresh blood 9.2 mm ± 3.3% ($n = 8$).

3.1.2. Influence of blood volume on blood spot weight

Lower weight was seen with blood spots of 10 μl: 2.02 mg ± 3.9%. The weight of blood spots, made with 20 and 30 μl was 3.77 mg ± 5.5% and 3.56 mg ± 4% ($n = 6$) respectively. However after application of 10 μl blood the paper disk was incompletely filled with blood. The disk was just filled with blood after application of 20 μl. Thirty microlitres filled the paper disk by a wide margin.

3.1.3. Influence of drying time on blood spot weight

Weight of the paper disks with blood spots became lower with longer drying times. The weight after 1, 4 and 18 h was respectively: 5.29 mg ± 6.1%, 4.16 mg ± 3.1% and 3.56 mg ± 4% ($n = 6$).

3.2. Method validation blood spot samples

3.2.1. Accuracy, precision, lower limit of quantification and detection limit

The precision and accuracy are shown in Table 1 and are within the 15% limit. The limit of detection was 0.26 μg/l.

The lower limit of quantification of the assay was 1 μg/l. The precision and accuracy at this level were 8.9% and 14.8%, respectively ($n = 5$).

3.2.2. Linearity

The calibration line was linear from 1 to 30 μg/l. The mean coefficient of determination of the calibration line was satisfactory: $R^2 = 0.9948 \pm 0.0029$ ($n = 5$). Higher concentrations than the upper limit of the calibration line were also measured: 50.0 and 100.0 μg/l. After extrapolation concentrations of 49.4 and 97.0 μg/l ($n = 2$), respectively were found.

3.2.3. Extraction recovery

The extraction recovery was: 78% ± 3.5% ($n = 6$).

3.2.4. Selectivity

No interfering peaks were observed in the blood from six tacrolimus-free routine patients.

3.2.5. Stability

3.2.5.1. Patients. Tacrolimus concentrations of the blood spot samples of patients were measured the day after sampling and again after several days storage under different temperature conditions. All concentrations were within the 15% limit (Table 2).

3.2.5.2. Calibration standards and quality controls. All tacrolimus concentrations of blood spot samples with calibration standards and commercial control were within the 15% limit during storage at 4 °C for at least 31 days (Table 3).

3.3. Comparison of tacrolimus concentrations of blood spot and venous samples of patients

The regression line according to Passing and Bablok is shown in Fig. 2. The 95% CI of the slope and the interval includes 1 and 0.

Bland Altman plots of the differences between venous and dried blood spot sampling are shown in Figure 3. There was no significant bias. All points were within or near 2 S.D. (±14%) from the mean difference. We consider this quite acceptable for clinical purposes.

Table 2
Stability of dried bloodspots samples of patients

Result 1 day after sampling $t=0$ (μg/l)	Result after storage (μg/l and percentage of concentration at $t=0$)	Storage condition	Storage time (days)
4.87	4.78 (98%)	Room temp	9
4.75	4.42 (93%)	Room temp	9
7.86	7.05 (90%)	Room temp	7
15.85	13.92 (88%)	37 °C	7
3.33	3.26 (98%)	37 °C	7
4.31	3.72 (86%)	37 °C	7
5.87	5.99 (98%)	37 °C	7
9.24	8.21 (89%)	37 °C	7
18.1	18.2 (100%)	70 °C	1
4.6	4.7 (102%)	70 °C	1
4.0	3.8 (95%)	70 °C	1
10.5	9.8 (93%)	70 °C	1
16.8	18.2 (108%)	70 °C	1
10.7	10.9 (102%)	70 °C	1
7.36	7.47 (99%)	−20 °C	8
12.67	13.56 (107%)	−20 °C	7
4.37	4.62 (106%)	−20 °C	7

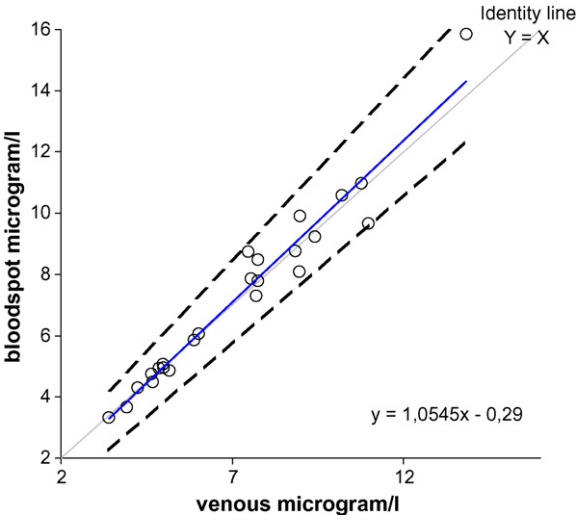

Fig. 2. Regression line of tacrolimus concentrations of patients measured with dried blood spot sampling and with venous sampling ($n=24$). Intercept = −0.29 (95% CI = −1.0006–0.237), slope = 1.055 (95% CI = 0.966–1.177).

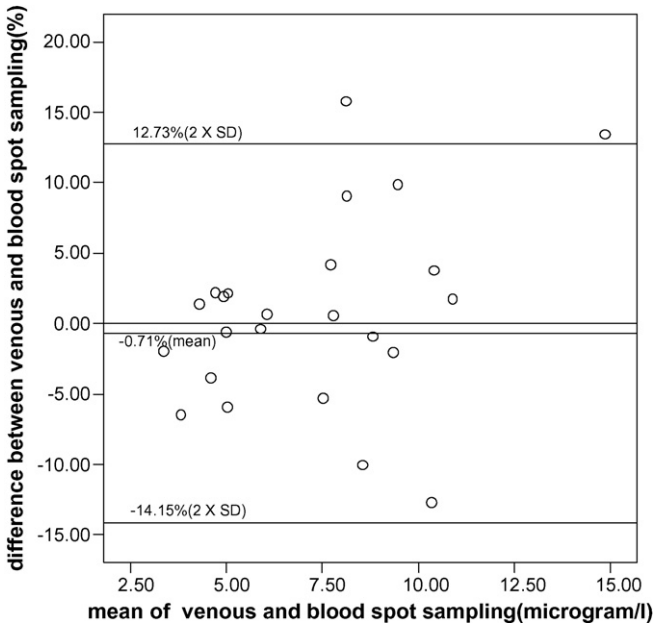

Fig. 3. Bland Altman plot of tacrolimus concentrations patients determined after venous and dried blood spot sampling ($n=24$).

4. Discussion

It was decided to use 30 μl fresh human blood for preparation of calibration standards and quality controls. Fresh human blood has closest likeness with fresh patient fingerprick blood. The decision was also based on the observations made in the preliminary studies. Thirty microlitres for calibration standards has also been used in other studies about therapeutic drug monitoring with dried blood spot sampling [6,7]. Application of 30 μl fresh blood assures thorough filling of disk with the blood spot and the diameter is approximately the same as blood spots produced by patients. Equal weight of punched out disks from blood spots made with 20 and 30 μl blood indicates that blood volume (between 20 and 30 μl) does not influence weight of the punched out disks and consequently the sample volume. The diameter, the weight of the spots, the colour on both sides of the paper and eventually the tacrolimus content seem to be determined by the type of blood. Differences of weight, diameter and colour were caused by difference in viscosity and diffusion properties. Viscosity of fresh blood is lower than haemolytic blood. Therefore

Table 3
Stability of dried blood spot samples of standards en ClinChek® controls

Standards and ClinChek® controls	$t=8$ days at 4 °C	$t=13$ days at 4 °C	$t=31$ days at 4 °C
Standard (0 μg/l)	<3	<3	<3
Standard (3 μg/l)	2.64 = 88%[a]	3.23 = 108%	2.91 = 97%
Standard (6 μg/l)	5.28 = 88%	6.40 = 107%	5.83 = 97%
Standard (9 μg/l)	8.60 = 96%	9.14 = 102%	8.31 = 92%
Standard (15 μg/l)	12.91 = 86%	15.53 = 104%	13.91 = 93%
Standard (30 μg/l)	27.49 = 92%	26.99 = 90%	28.87 = 96%
ClinChek®-1	4.28 = 87%[b]	5.51 = 112%	5.07 = 104%
ClinChek®-2	4.69 = 96%	5.17 = 106%	

[a] % of nominal concentration of freshly prepared calibration standards.
[b] % of measured concentration at $t=0$: 4.92 μg/l.

the permeation of fresh blood in the paper is complete, there is less spreading and a smaller diameter and eventually a higher weight of the punched out disk.

For drying of the blood samples a long drying time, overnight drying, was chosen, because we wanted to be on the safe side. Actual sampling time of the blood spots is short and comparable with venous sampling. However we do not think the drying time will be a problem with routine monitoring. Moreover in daily practice postal transport takes also much time: at least 24 h.

The commercial ClinChek® control does not consist of fresh blood. Higher viscosity diminishes permeation of the paper and alters flux and diffusion resulting in blood spots with larger diameter. The punched out dots have lower weight and contain less tacrolimus. This explains the results of this quality control being more than 15% lower than specification. ClinChek® quality control could nevertheless be useful (after concentration correction) as quality control, because the deviation is constant and the coefficient of variation is small. For satisfactory results thorough filling of the predrawn circle on the filter paper with sufficient (30 μl or more) fresh and not hemolytic blood, is important. Therefore visual control of samples in the laboratory before analysis is advisable. Criteria are: complete filling of the predrawn circle, symmetric spreading of the blood spot around the centre and evenly dark coloured on both sides of the sampling paper.

Dried blood spot samples are stable at a wide variety of temperature. The stability study indicated that batchwise preparation in advance of dried blood spots with quality controls and calibration standards is feasible. Storage of dried blood spot samples of patients is possible for at least 1 month at 4 °C.

In a recent article very low hematocrit concentrations have been reported to influence dried blood spot measurements of amino acids with neonates [15]. We found a good correlation between the patients results of venous and blood spot sampling. However our patient population consisted of stable adult outpatients. The hematocrit of these patients was not very deviant: 0.40 ± 0.06 ($n = 14$). Our blood spot method has not yet been investigated with hospitalized or unstable patients and patients with low hematocrit. Therefore the influence of low hematocrit is not known yet and will be the subject of future investigation. In the same article also a significant difference of the concentration of amino acids in disks from peripheral and central punches was mentioned [15]. However the authors filled a large circle (15 mm diameter) with blood and made 3 mm diameter punches: 4% of the circle surface. We used 8 mm circles and punched 7.5 mm disks: 88% of the surface. Therefore the difference between peripheral and central locations with our method was small.

5. Conclusion

In this preliminary investigation a dried blood spot method for the assay of tacrolimus has been described. The method seems promising for monitoring of stable outpatients.

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