Dried blood spot methods in therapeutic drug monitoring: methods, assays and pitfalls

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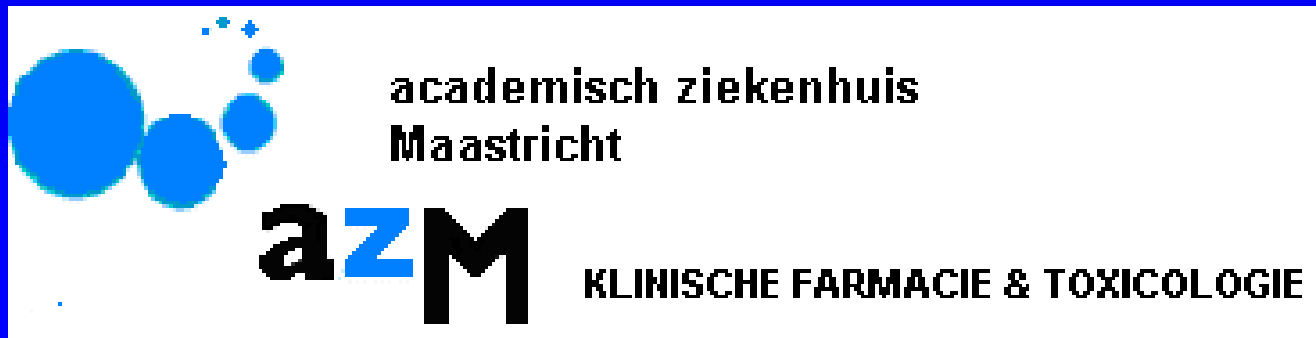


IATDMCT

International **A**ssociation for **T**herapeutic **D**rug **M**onitoring and **C**linical **T**oxicology founded in 2007

Scientific committee: *"New sampling Strategies" with special interest in dried blood spot method*

Chair Leo M Stolk

information: www.iatdmct.org see scientific committees

Contents of this presentation

1) DBS drugs assays available
2) Sampling technique
3) Relevant factors/co-variates
4) Conclusions

Also examples our own experience with DBS will be discussed

Blood spot method in TDM

- Use of blood spot method in TDM has been developed after the neonatal method.
- Since the eighties, but especially during the last years many assays based on blood spot have been published: > 40 papers.

Available Drugs assays for TDM based on DBS

Dried Blood Spot Methods in Therapeutic Drug Monitoring: Methods, Assays, and Pitfalls

Peter M. Edelbroek, Jacques van der Heijden, and Leo M. L. Stolk. Ther Drug Monit Volume 31, Number 3, June 2009

review of 30 papers about TDM based on DBS

Assays developed in our laboratory

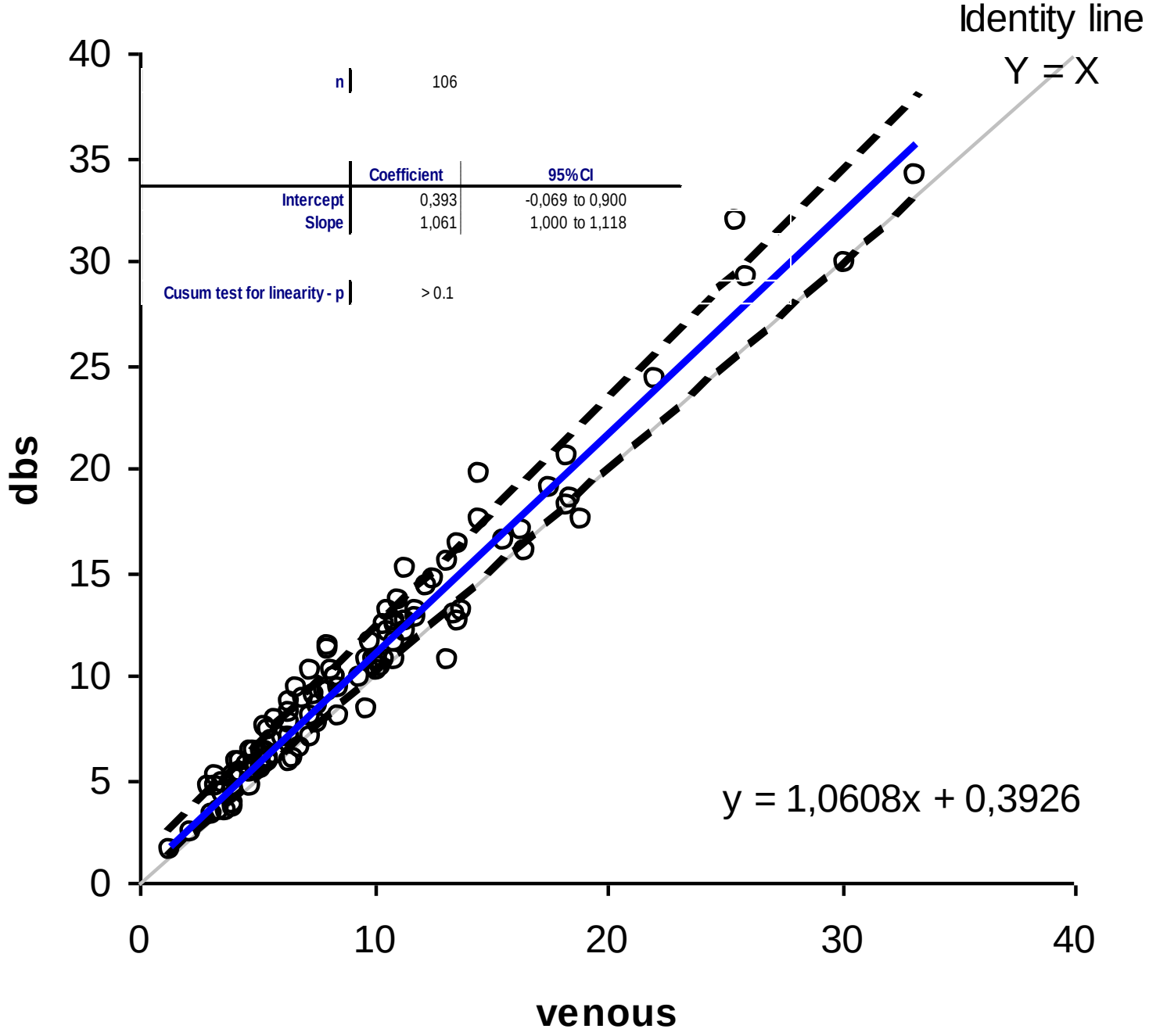
- Hoogtanders K, van der Heijden J, Christiaans M et al Therapeutic drug monitoring of tacrolimus with the dried blood spot method. J Pharm Biomed Anal 2007;44:658-664.
- Van der Heijden J, de Beer Y, Hoogtanders K, et al . Therapeutic drug monitoring of everolimus using the dried blood spot method in combination with liquid chromatography-mass spectrometry. J Pharm Biomed Anal 2009;50:664-70
- Hoogtanders K, van der Heijden J, Christiaans M, van de Plas A, van Hooff J, Stolk L. Dried blood spot measurement of tacrolimus is promising for patient monitoring.Transplantation 2007;83:237-8.

Blood spot TDM assays

- Antiepileptics (Dr P Edelbroek)
- Antimalarials (Dr Y Berqvist)
- Antibiotics
- Antiretrovirals (Prof V Kaever)
- Immunosuppressives (Dr L Stolk, A Wilhelm)
- Miscellaneous (metformin, paracetamol, theophylline)

Validation

- In nearly all papers analytical validation seemed adequate.
- Clinical validation was sometimes lacking: comparison of concentrations in venous samples and DBS samples drawn at the same time.


Identity line
Y = X
n 106
Coefficient 95% CI
Intercept 0,393 -0,069 to 0,900
Slope 1,061 1,000 to 1,118
Cusum test for linearity - p > 0.1
y = 1,0608x + 0,3926
dbs
venous
0
5
10
15
20
25
30
35
40
0
10
20
30
40

Stability

- Stability was investigated in most studies.
- Few examined the whole range of temperature conditions that could be expected during shipment.
- However most analytes were reported to be rather stable.

Tacrolimus stability in blood spot

- No deterioriation of blood spot concentrations of blood spots reference standards during at least 31 days at 4°C.
- Blood spots of patients: no deterioriation after 7 days at –20° C, Room temperature, 37°C and one day at 70 °C.

  NB temperature postal box in summer!!!

Sampling Techniques

- Patient cuts himself with an automatic lancet
- Patient fills a predrawn circle (8 mm ø) on filter paper completely with blood
- The blood spot must dry for at least 3 hours
- The paper with the sample is sent by mail to the laboratory.

Blood spot method A

In the laboratory:

-Quality control of the blood spot.

- a disk (7.5 mm ø) is punched out

-Extraction of the disk takes place

-Assay

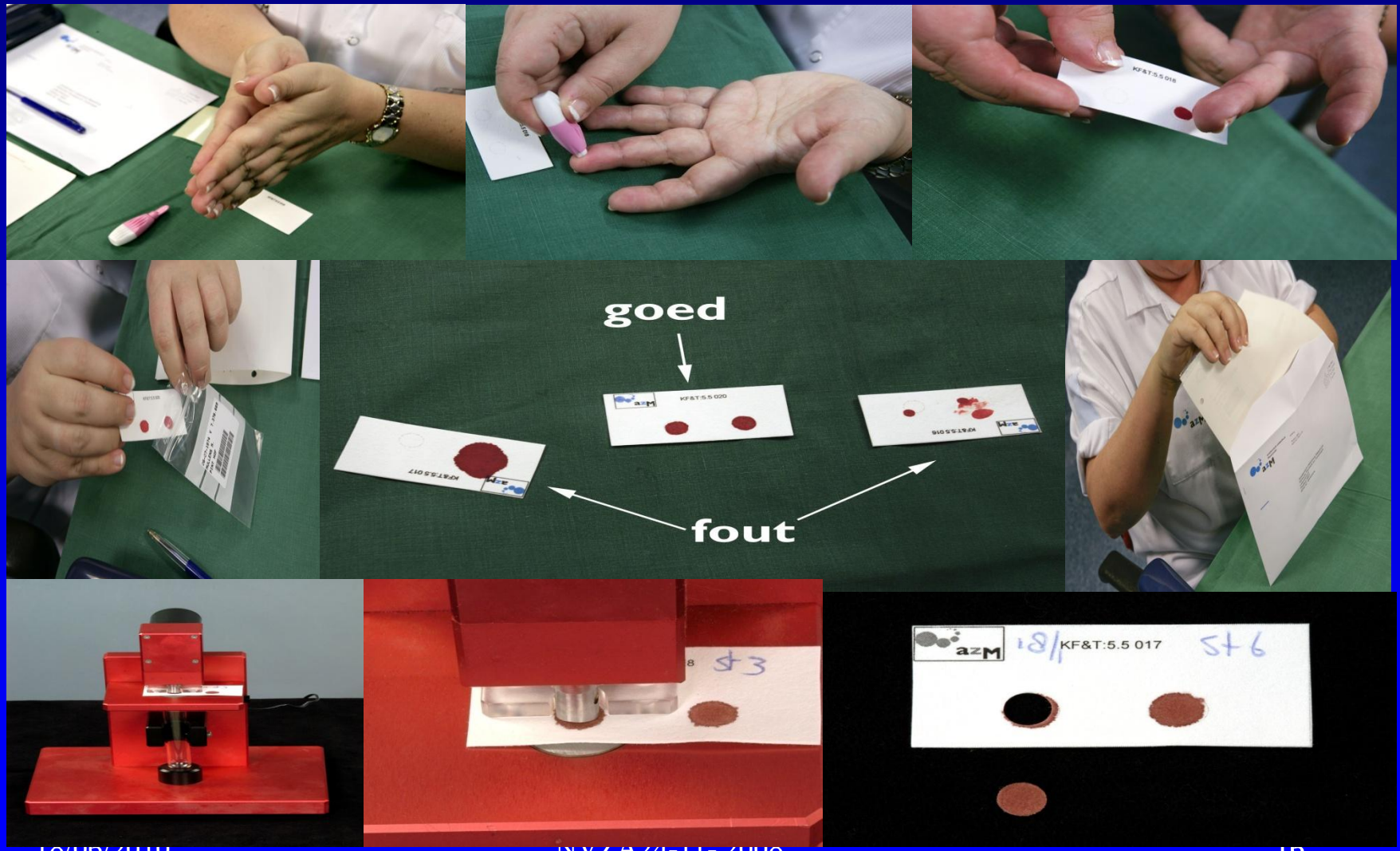
Alternative method B

- A finger prick is made
- Patient or trained technician brings an accurate volume of blood on the sampling paper with a pipette.
- In the laboratory the whole spot is extracted.

Blood collection in detail

- Single use lancet devices (2 mm needle depth or other)
- Warming and cleansing before puncture.
- Disinfection with 70% isopropanol
- Drying
- Discard first drop (dilution tissue fluid)
- No milking or sqeezing allowed

Blood spot in practice



Advantages DBS in TDM

-The patient is able to sample at home (after adequate training)

-Concentration time curve

- Postal mailing is simple (biohazard risk is negligable)

-In the dry matrix drugs are stable

-Small sample amount: ± 30 µl per spot.

Disadvantages

- Sampling is not under control
- Despite training quality some samples is unsatisfactory in our experience.
- Some patients afraid of finger prick
- Small sample amount: expensive analysis. Usually only one drug assayed per sample
- No spare sample after analysis

Relevant factors/co-variates

1) Sampling (filter) paper:

Paper Properties like

- Pore size
- Thickness
- Basis weight

  determine loading capacity and spreadability of whole blood on sampling paper

Medical device

- In many screening programs Whatman 903 specimen collection paper is used, a Food and Drug-administration registered in vitro class II medical device, with the CE mark of the European Union.

Sample handling and drying

- Medicines are handled by the sampler: therefore sample contamination risk should be minimized.
- It is important to dry DBS samples completely before transport. Drying time should be at least 3 hours at 15-22 °C over an open surface.

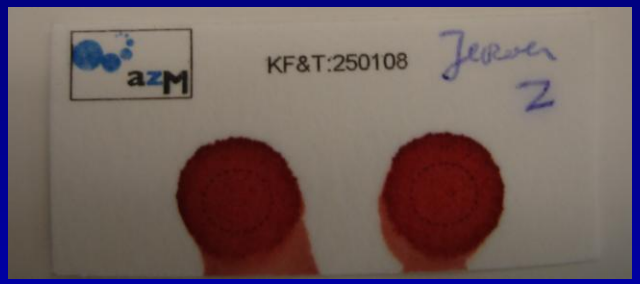
Packaging and transport

- DBS samples can be sent via normal postal systems without special mailing cartons

  Knudsen R et la *Guidelines for the shipment of dried blood spot specimens.* Atlanta. Center for disease control and prevention 1995.

- Specimens can be protected against humidity and moisture by being packed in paper or plastic bags.

Problem in our lab



Sometimes bloodspots were received as a smear

-For some patients adequate drying proved too complicated

-Solved by routinely adding of a drying agent.

Inspection of DBS samples

- Visually checking the DBS samples on arrival is advisable. Ensuring that:
- The predrawn circle is completely filled
- Blood spot is spread symmetrically around the center
- Even dark colouring on both sites paper

Extraction methods

- In our experience drugs should be extracted with methanol, acetonitrile or a mixture of both. Using these agents protein denaturation and precipitation also occur.
- Using water increases interference with endogenous components.
- In case of low recovery, impregnation of the sampling paper could facilitate extraction

Hematocrit

Dried blood spot method uses blood.

What about influence hematocrit?

Reports of hematocrit (HT)

Acetylcarnitine conc were higher with higher HT (Holub 2006)

- With MPA with higher HT, > 10% deviation. But no deviation of ciclosporin conc in HT range 0.2-0.72 (Wilhelm 2009)
- We found no difference of tacrolimus concentrations in the range from hematocrit 0.32-0.49.Outside this range we did.

Preparation of calibrators

- The use of fresh normal blood for calibration standards is essential, especially for the DBS method A using the paper disk as a volumetric measurement.
- Hemolyzed blood or blood with a deviating hematocrit should not be used.

Questions for future investigation

- Automation
- Direct analysis in paper with desorption electrospray ionisation MS
- Covariates like Hematocrit, protein binding
- Program for proficiency testing blood spot
- Development of more drugs assays in blood spot. Preferably all assays, which are needed for a patient, also non TDM assays (eg creatinine)

Conclusions

- Blood spot can be useful alternative for venous blood analysis.
- Blood spot method is a new chance for TDM
- For every application advantages should be weighed against potential errors, which could be introduced with the DBS method.