An overview of the implementation of DBS at Novartis: results and next step

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On behalf of

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Agenda

- Introduction
- Compounds tested and method(s)
- Overall assessment & outcome
- Next step
- General consideration
- Conclusion

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Agenda

Introduction

Compounds tested and method(s)

Overall assessment & outcome

Next step

General consideration

Conclusion

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Introduction

- Emerging sampling technique for pharma R&D
- Widely used in new born screening (since 1963), TDM, infectious disease diagnostics & nucleic acid analysis.
- Human PK application in TDM since 1986 (Hibberd et. al.). First publication on pharma application in 2004.

Introduction

- Big pharma (and many others) found DBS of potential use

As of Dec 2009...just an excerpt*:

- **Sanofi-Aventis**: Using since 2005 for anti-malarial drugs. Implemented in drug development since Apr-2009. Tested with 34 compounds. Applied to 26 compounds. 2 Phase-I studies in 2009
- **GSK**: Tested DBS since 2006. Implemented in 2008. Applied to 83 compounds. Validated 143 methods. 5 phase-I studies
- **Pfizer** applied DBS to 50 compounds
- **Astra Zeneca**, **BMS** and **Abbott** have successfully tested DBS
- **Covance** and **Harlan** applied DBS to 10 and 17 compounds, respectively.
- **Novartis:** Proceeded cautiously to investigate important issues: sensitivity (LLOQ), homogeneity, HA compliant BA method, automation, handling of unstable metabolites with DBS, possibility of mixed matrix for analyte and/or metabolites and interpretation of plasma data from research

*: source: mainly EBF Barcelona meeting, December 2009

Introduction

- Novartis status:
  - Gathering information since 2008
  - Commenced with evaluations in 2008
  - Performed in-depth assessments for 13 different compounds between 2009-2010.
  - Initiated DBS sampling/analysis in two clinical studies, several TK studies and a mouse PK study
  - An application note has been published
    - S. Buckenmaier, C. Emotte et al, 2008.
  - A review paper has been published
    - W. Li et al, Biomed Chromatogr. 2010; 24: 49-65

Agenda

Introduction

Compounds tested and method(s)

Overall assessment & outcome

Next step

General consideration

Conclusion

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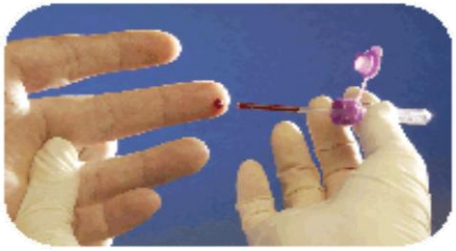
Compounds tested from 2008 to 2009: Overview

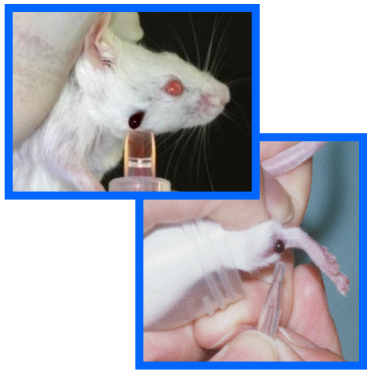
Cpd	MW (g/mol)	PKa	Clog P	R&D Phase
1	486	4.3	5.8	Non GLP tox study in dog
2	414	3.3	-0.1	Non GLP tox study in mouse
3	561	2.8	4.2	Non GLP tox studies
4	455	4.1	6.8	Clinical and preclinical studies
5	441	4.9	4.5	Non GLP tox studies
6	413	3.8 / 9.5	4.8	Non GLP tox studies
7	349	7.6 / 9.3	2.6	Non GLP tox studies
8	1202	n.a.	14.4	Clinical study
9	520	4.2	3.7	Non GLP tox studies
10	444	4.1 / 10.7	> 4.1	Non GLP tox studies
11	500	3.7	4.7	Non GLP tox studies

A wide variety of compounds has been tested having different Physico–Chemical properties

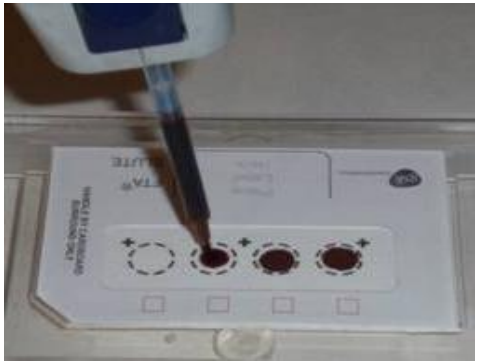
Method: Sample collection

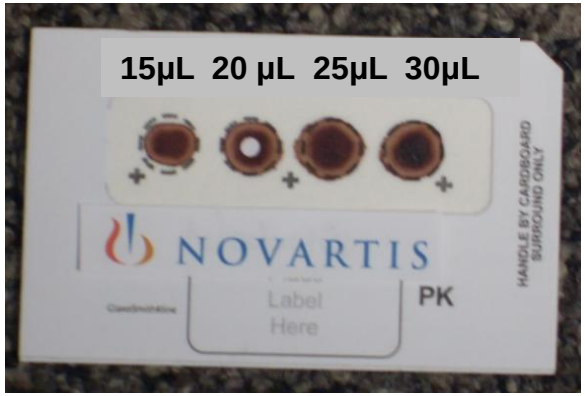
Sample collection





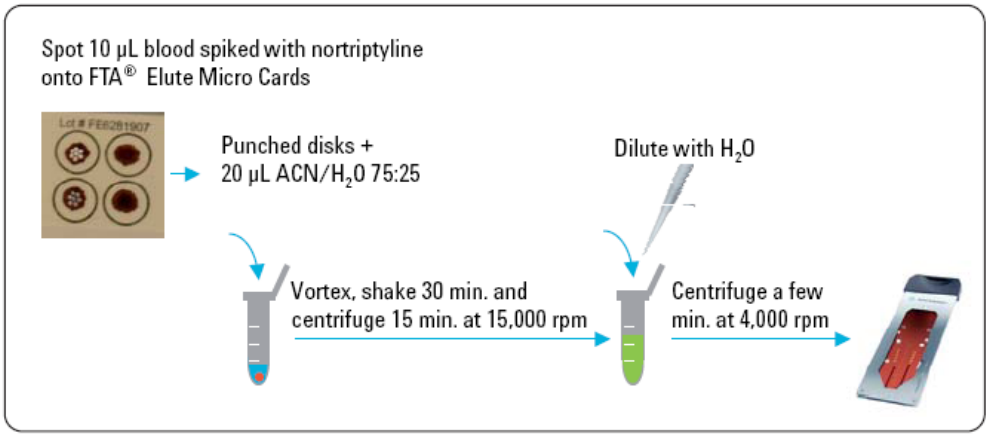
Adding blood spots



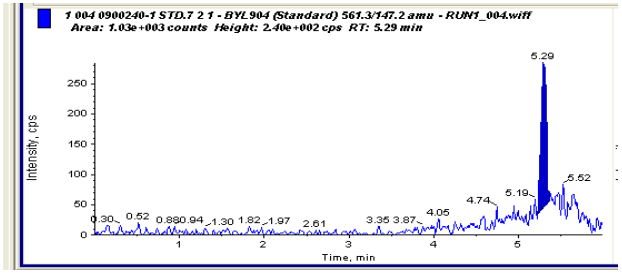


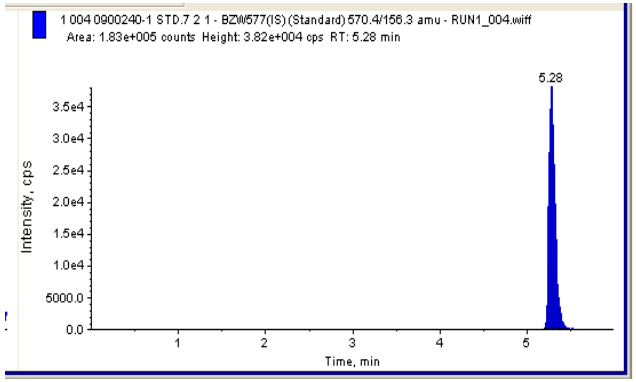
- Cards are air dried & stored/shipped desiccated at room temperature
- Discs are punched out of the DBS card for quantitation
- Both coated and uncoated
- Coated DBS card matrix
  - *Lyses cells*
  - *Inactivates pathogens*
  - *Denatures blood proteins and enzymes*

Method: Sample processing



LC-MS/MS Analysis





LC-MS/MS chromatograms

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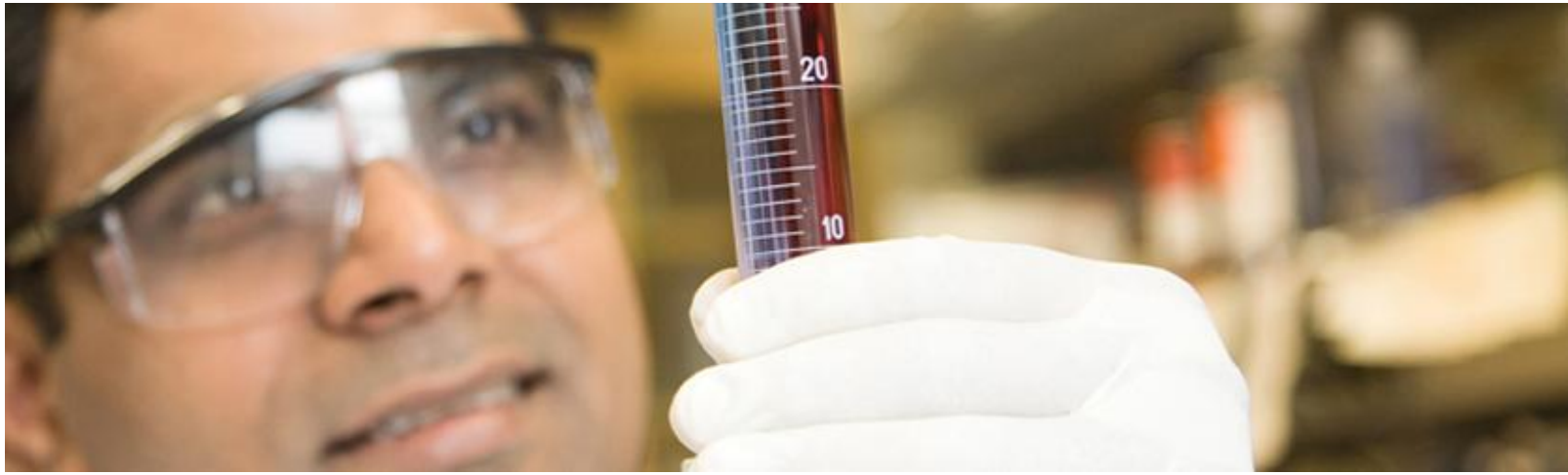
Agenda



Introduction

Compounds tested and method(s)

Overall assessment & outcome

Next step

General consideration

Conclusion

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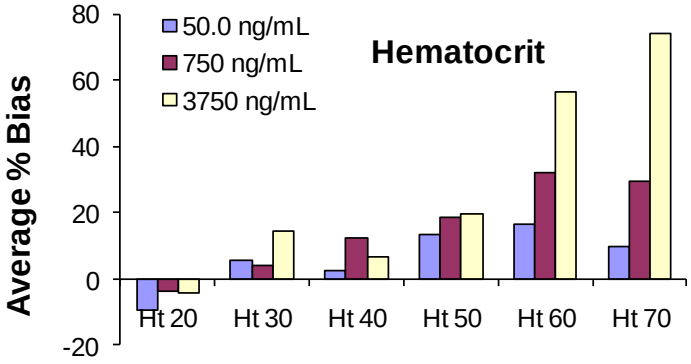
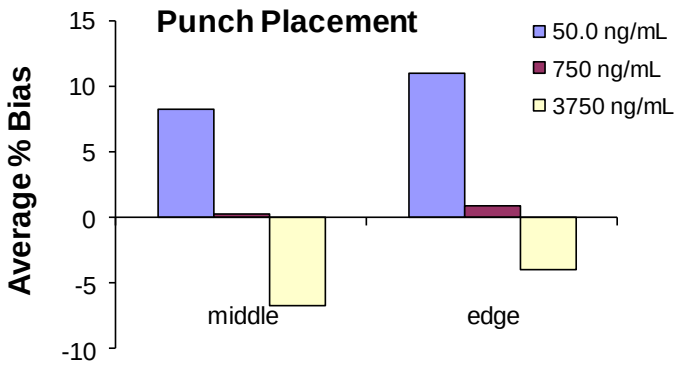
Assessment

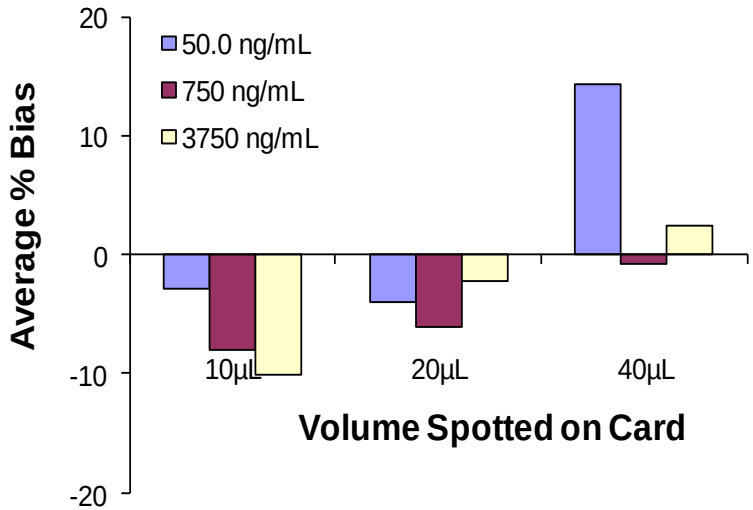
- In general, for most of the compounds tested the following parameters were assessed:
  - Selection of the DBS card
  - Sensitivity, Selectivity
  - Spot homogenity
  - QC validation
  - Stability at room temperature
  - Selection of the solvent of extraction and method recovery
- Most importantly the DBS outcomes were compared with results obtained using the conventional sampling strategy
- Some additional evaluations have been conducted (e.g. hematocrit, blood volume, drying time....)

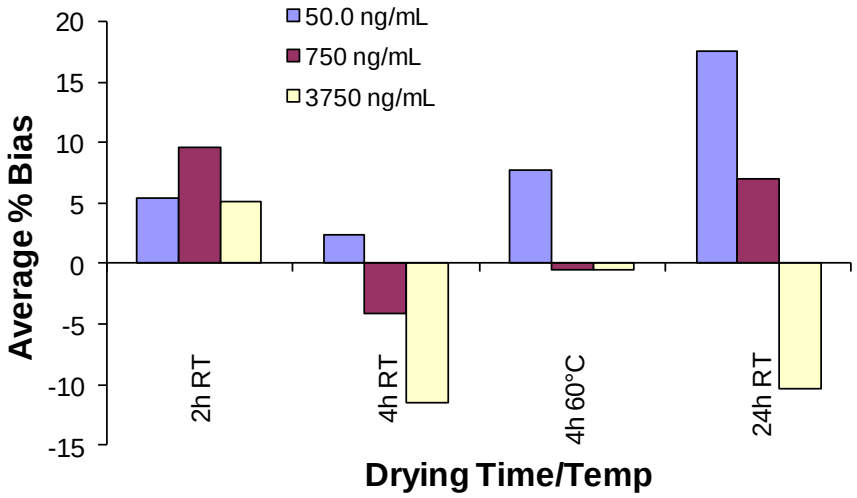
Assessment: some specific examples

- Impact of hematocrit, blood volume, punching location and drying time on the accuracy of determination with Cpd 8









Assessment: some specific examples

- Stabilty with Cpd 7 in rat blood
  - Whereas most analytes may be stable using DBS, unstable compounds present a challenge for DBS sampling as enzyme inhibitors can not be easily mixed during sample collection.

w/ inhibitor in whole blood

QC Conc (ng/mL)	Mean % Bias
7.50	13.1
100	-0.3
375	20.0

w/o inhibitor in DBS

QC Conc (ng/mL)	Mean % Bias
7.50	-77.7
100	-64.2
375	-64.0

Overall outcome

- In the majority of the cases, the results of our assessments were within our acceptance criteria (FDA guidance on method validation)
- The results found with DBS for most of the compounds were in agreement with those obtained using the conventional sampling method
- Advantages
  - Cost savings and convenience, Less invasive, Convenient for low volume sample collection, Enable juvenile toxicology studies, Convenient collection, drying, storage & shipping methods, Blood spot is homogeneous for volumes ≤ 20uL, Centrifuges or freezers not required at the collection site (cost saver), Easy extraction. No need for SPE plate and evaporation (cost & time saver), No liquid to freeze, No F-T damage to analytes (time saver).....
- Possible hurdles
  - Cannot be used for volatile and air sensitive compounds, Ultra-low LLOQ (pg/mL) may be limited, Photo sensitive compounds require special handling, Lack of reliable automation (robots: BSD1000 & BSD600 DUET gene punches), May not be applicable for some unstable metabolites....

Agenda

Introduction

Compounds tested and method(s)

Overall assessment and outcome

Next step

General consideration

Conclusion

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Next step

- For new compounds:
  - Sampling using both DBS and whole blood will be performed in animals as part of the DRF studies in each species
  - If the exposure data are identical within the acceptance criteria, DBS will be used as the sampling method for the subsequent GLP studies in the same species
  - Complete method validation will be performed according to HA Guidance
  - The DBS sampling method will be implemented in the subsequent clinical studies
  - Exposure data for compounds unsuitable for DBS application will be obtained using whole blood or plasma (for sensitivity) as the matrix

Next step

- DBS will be implemented for one project currently in Phase IV in order to prepare an upcoming study in neonates.
- In the frame of a new initiative 12 other marketed compounds will be evaluated using DBS
- Further investigations in projects with unstable metabolites will be conducted (e.g. N-oxide)
- Robotic systems: (BSD600 DUET has been evaluated) other automation will be evaluated

Agenda

Introduction

Compounds tested and method(s)

Overall assessment and outcome

Next step

General consideration

Conclusion

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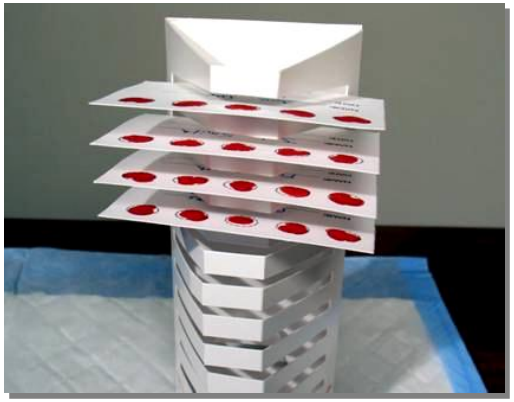
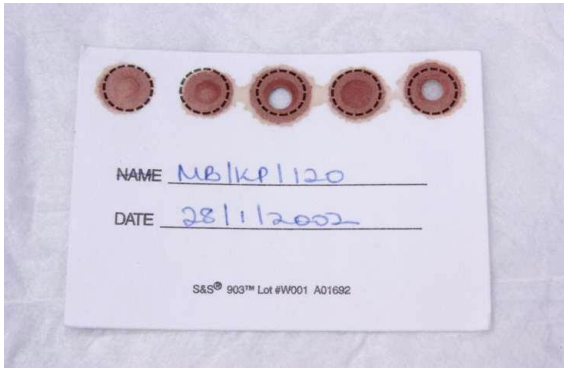
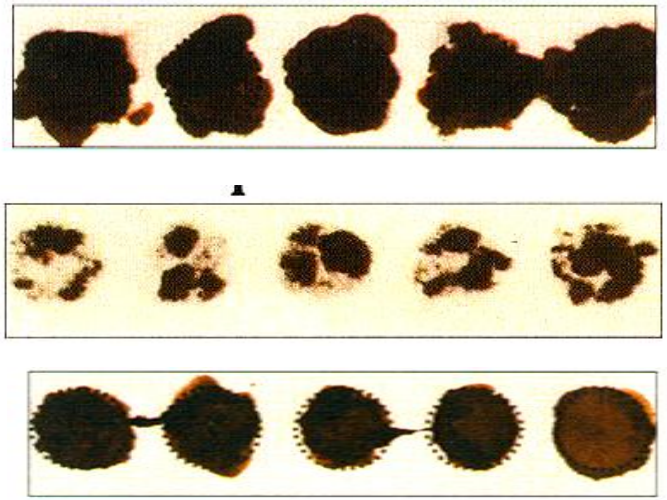
General consideration

- From a GLP to a GCP environment







General consideration

- Further validation required ?
  - Effect of Hematocrit.
    - Since it is directly proportional to the viscosity of the blood, it may affect flux and diffusion properties of the blood spotted on the card
      - » *Concentrations were found to be significantly higher in samples with high hematocrit and lower in those with low hematocrit (Adam et al 2000, Mei et al 2001, Holub et al 2006)*
      - » *It seems however to be compound dependent since no effect has been observed in other investigations (Newman et al., 2009, Wilhelm et al., 2009)*
  - Influence of blood spot volume
    - Does the blood volume need to be accurately controlled ?
      - » *Mean results (HPLC) of the measured phenylalanine concentrations from 35 µL spots was lower than that of 100 µL spots (Adam et al., 2000)*
      - » *In contrast recent publications did not show any effect (Spooner et al., 2009, Liang et al., 2009)*
  - Influence of the punch placement
    - Are the measured analyte concentrations identical between the central and peripheral area within the same spot ?

General consideration

- Influence of the spotting device
  - Pipet or capillaries
    - Is there an expectation to have a calibrated pipet in a GCP environment (especially PhIII)?
- Site for sampling collection
  - Is comparable PK data obtained for samples collected from a venous cannula compared to those from fingertip or heel prick ?

Agenda

Introduction

Compounds tested and method(s)

Overall assessment and outcome

Next step

General consideration

Conclusion

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Conclusion

- In the majority of cases, results from our assessments were within the established acceptance criteria (FDA guidance on method validation) and most importantly, the results were comparable to data obtained using conventional blood sampling techniques.
- DBS is viewed to be a very promising and convenient approach to replace conventional whole blood sampling.
- Ideally, DBS should be implemented as early as possible during drug development and the project should then continue using DBS.

However

- Some limitations (e.g. low pg LLOQ, unstable compound, lack of automation...) may restrict its use

In addition

- Validation activities may require adjustment when using DBS and some assessment(s) may not be required (e.g. F/T cycle)

Conclusion “take home message”

Acknowledgment

- All BA folks involved in the DBS implementation globally
- Francis Tse
- Olivier Kretz
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