Feedback From the 2011 APQ Open Forum

DBS and Microsampling: Moving Past the Hype to Knowledge and Implementation

Eric N. Fluhler, Pfizer, on behalf of AAPS Bioanalytical Focus Group

DBS and Microsampling

- Past 3 – 4 years, an explosion of research and interest in DBS and microsampling techniques
- Senior management has heard the buzz
  - On top of 3 R's, pressure to implement in the face of constrained budgets
- Bioanalytical Focus Group sought a mechanism to bring scientific and regulatory issues to the forefront and explore potential of future technologies
  - DBS Webinar, Educational Emphasis
  - APQ Open Forum at Annual Meeting, hear emerging positions from industry and regulators and engage in open discussion

DBS Webinar

- Bioanalytical Focus Group hosted a webinar in September

  "Dried Matrix Spot Bioanalysis: Current Status and a Primer for APQ 2011 Open Forum"
- Hosted and coordinated by Steve Lowes
- DBS Webinar Expert Panel:
  - Chris Evans, Shane Needham and Qin Ji
- Participation:
  - Over 170 people registered
  - Over 100 sites participating from 6 countries with 165 participants
  - Scheduled for a 30 min presentation and a 30 min discussion, the discussion lasted over 50 minutes and there was still not time to get all of the questions answered!

APQ Open Forum Agenda

- Use of Dried Blood Spots for Measurement of Therapeutic Antibody Concentrations – State of the Technology
  - Marc Retter, Amgen
- Microsampling: DBS, Whole Blood, and Plasma
  - John Dunn, GSK
- Dried Blood Spots – Pharmacokinetic Aspects and Interpretation
  - Delphine Valente and Patricia Zane, Sanofi
- Perspectives on Micro Sampling Dried Blood Spots
  - CT Viswanathan, Formerly FDA
- Open Discussion

Use of DBS for Measurement of Therapeutic Antibody Concentrations – State of the Technology

Marc Retter, Amgen

- Internal efforts focused on application to in vivo Discovery samples (ELISA)
  - Case studies in rat and monkey (5 mAbs)
  - DBS provided similar t1/2 values as serum/plasma
  - AUC consistently lower by DBS relative to serum/plasma
  - Demonstrated limited LTS for some compounds (> 2-3 mo.)
- Evaluation of Hct and recovery role in results
  - Spiked plasma, plasma & RBCs from spiked blood, DPS and DBS
  - ELISA and ICP-MS

Use of DBS for Measurement of Therapeutic Antibody Concentrations – State of the Technology
Marc Retter, Amgen

- Differences in plasma/serum and DBS PK
  - Hct and blood partitioning
  - Recovery (lower for DBS vs DPS)

Take-Home Message

- Generally optimistic about future of technique
- Rapid evolution taking place
- Needs further development before it's ready for regulated studies on protein therapeutics (e.g. B/P, automation, card types)

Microsampling: DBS, Whole Blood, and Plasma

John A. Dunn, GlaxoSmithKline

- Reviewed *state of the art/science* for DBS
  - Cards
  - Hematocrit
    - Age, gender , disease population
    - Spot size and homogeneity (^{14}C labeled compounds)
    - Needs thorough assessment to understand impact for study population
    - Solutions: sample entire spot (requires accurate spotting)
  - Real World Application and Outcomes
    - DBS regulated toxicology program submitted
    - FIH program to include DBS and blood/water for all subjects/time pts
    - Demonstrated excellent concordance between methods
    - Submitted plan for future BA work to agency

    Agency Feedback: Continue to compare methods during development. Sufficient #'s for comparison.

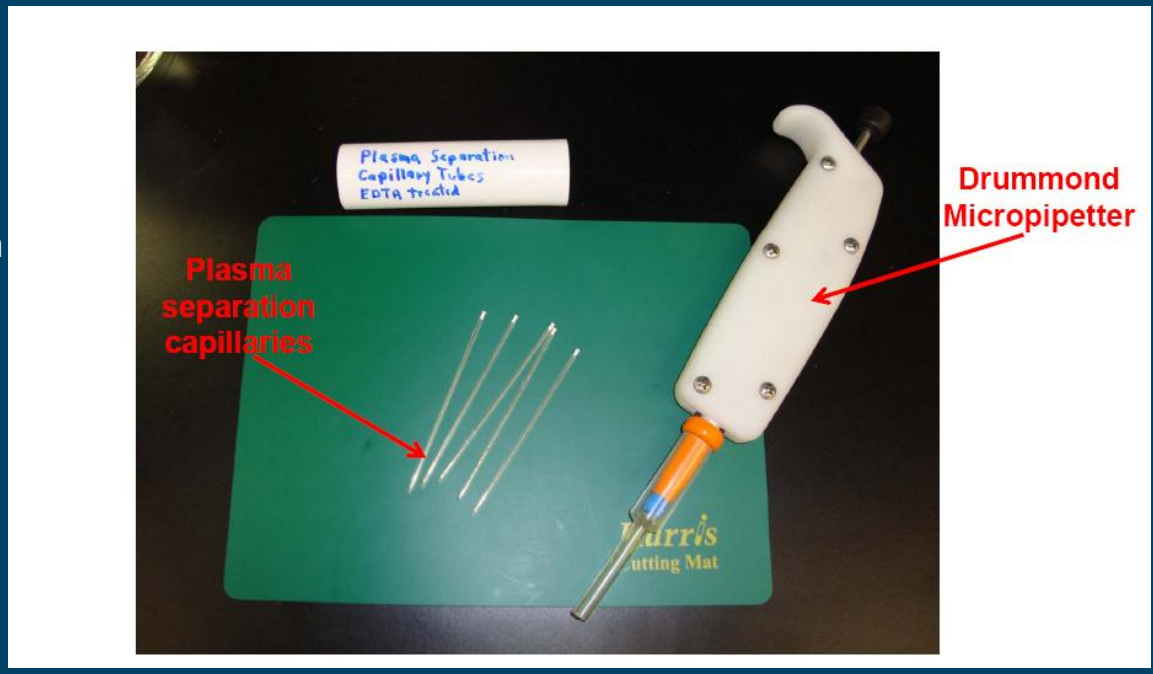
Microsampling: DBS, Whole Blood, and Plasma

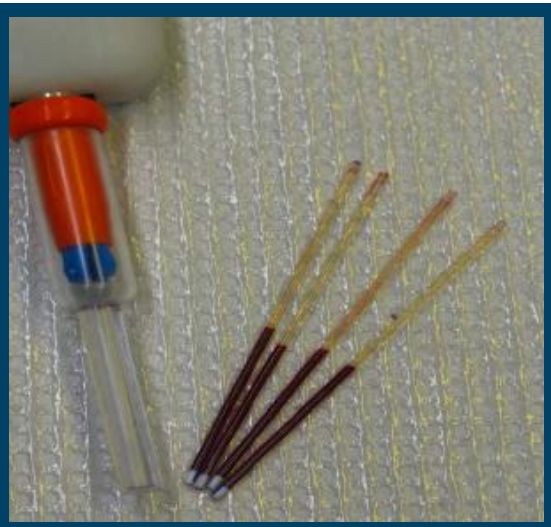
John A. Dunn, GlaxoSmithKline

- Reviewed state of the art/science for *'Wet' Microsampling*
  - Current Challenges
    - Collection: capillaries, accurate volumes
    - Labeling
    - Matrix transfers and automation
    - Matrix separation (plasma)
    - Training of clinical staff

Plasma Microsampling

- **Collaborated with Drummond to design prototype device capable of microsampling wet plasma**
- **Utilizes capillary tubes wrapped in Mylar®**
  - Tubes are treated with anti-coagulant (EDTA)
  - Porous end plug that allows capillary action & dispense
  - Thixotropic gel is added to separate plasma and RBC
  - Separation based on density differences between RBC and plasma upon centrifugation
- **Accurate plasma volumes dispensed by the device**





0.5 mL Micronics™ sample tubes

Slide courtesy of John Dunn

Microsampling: DBS, Whole Blood, and Plasma

John A. Dunn, GlaxoSmithKline

- Whole Blood Feasibility Pilot (in vitro)
  - Aliquoted whole blood (50 uL) from bulk
  - IS in methanol/water (25 uL), incubation, PPT w/acetonitrile
  - Good linearity, P/A, F/T
  - Currently working with lower volumes
- Plasma Microsampling Pilot (Acetaminophen dosed rats)
  - 75 uL blood into EDTA coated mylar wrapped capillaries
  - Centrifuge and pipette plasma to micronic tubes
  - Extract 5-10 uL plasma
  - Excellent concordance between conventional plasma assay and plasma microsampling (no statistical differences in PK parameters)

Dried Blood Spots – Pharmacokinetic Aspects and Interpretation

Patricia Zane and Delphine Valente, Sanofi

- Application of DBS at Sanofi
  - ~76 compounds tested, all phases of development
  - <20% failure rate, failures mostly due to inadequate human LLOQ
- Detailed bioanalytical methodology selection strategy
  - Discovery: fixed volume DBS ; in vivo B/P (time, [drug] dependence) in pharm and tox species
  - Preclinical: Stability and LLOQ, HCT effect (std or fixed volume DBS), B/P in vitro/vivo correlation across species (DBS only or DBS + plasma in clinic), concentration dependence for f_u
  - Clinical: Venous vs. peripheral sample comparisons (selection of go forward sampling site for phase II/III)
  - Transitions from plasma to DBS in various phases

Dried Blood Spots – Pharmacokinetic Aspects and Interpretation

Patricia Zane and Delphine Valente, Sanofi

- Detailed bioanalytical methodology selection strategy cont.
  - Transitions from plasma to DBS in various clinical phases
- Areas for improvement
  - Automation, impact of physiological conditions (HCT), papers or devices to overcome obstacles
- Real World Application and Outcomes
  - Feb 2011 Sanofi received approval for first IND with blood only as matrix, no comments.
- "Dried blood spot samples are in essence the same as whole blood samples and have most the same issues as those of the matrix concerning estimation and interpretation of PK parameters"

Perspectives on Microsampling Dried Blood Spots

CT Viswanathon, Formerly FDA, CT Viswanathan and Assoc. Inc.

General Impressions from Overview

- Microsampling consistent with agency goals to align with advancement of science
- Agency acknowledges potential advantages of technology over conventional sampling and future is bright but…….
- Not clear yet exactly what data are needed to establish the acceptability of data
- Overall method needs further refinement and resolution of issues
- No serious regulatory discussion has been conducted to date

Perspectives on Microsampling Dried Blood Spots

CT Viswanathon, Former FDA, CT Viswanathan and Assoc. Inc.

Remaining Issues

- Homogeneity of spot, dilution integrity, ISR, etc.

So are we ready to make significant decisions from DBS data?

- Probably not, and particularly where BE is concerned
- Fit for purpose applications for various phases of development seems appropriate provided proper documentation is provided
  - Validations must be robust and address above issues
  - Correlative studies needed
  - Preclinical and pediatric applications referenced

Open Forum Discussion Points

- Much discussion around Sanofi decision tree and desire to have it published and test success across companies
- Discussion on transitioning methods (DBS to plasma and plasma to DBS, within and across species). Supporting package must allow translation.
- For fixed volume samples, need to document pipette/device P/A and training for users
- How to best deal with HCT differences during application
- Documentation of conditions during shipment critical (data loggers)
- Experience is that ISR has been OK
- Microsampling is in its infancy and engineers need to find better ways to pipette and automate process

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