Dried Blood Spot (DBS) Testing & the Potential for the ABP

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The Typical Athlete Experience
Current Blood Testing Strategies

- **WHOLE BLOOD**
  - Complete Blood Count (ABP parameters)
  - ESA Testing

- **SERUM**
  - Growth Hormone Testing (isoforms & biomarkers)
  - ESA Testing
What is a Dried Blood Spot (DBS)?

- A form of biosampling where blood samples are blotted and dried on filter paper. The dried samples can easily be shipped to an analytical laboratory and analysed using various methods such as mass spectrometry.
What Prohibited Substances can be Detected?

- **Direct detection:** anabolic agents, peptide hormones, beta-2 agonists, hormone and metabolic modulators, diuretics, stimulants, narcotics, cannabinoids, glucocorticoids - due to volume limitations, an abbreviated menu of WADA Prohibited List
- **Direct detection:** testosterone esters
- **Indirect biomarkers:** ABP parameters
- **Indirect biomarkers:** IGF-1 & Fibronectin 1
- DNA analysis
- New biomarkers

**Important:** Window of detection is different in blood vs. urine
Now & the Future...

**Venous Blood**
- Phlebotomist
- Venipuncture
- Collection of whole blood or serum
- Volumes are generally between 3-5mL
- Strict transportation requirements
- Storage frozen possible only for serum
- Many billions of samples collected globally annually as clinical samples
- WADA Blood Collection Guidelines
- Analyses fit-for-purpose with existing methods and instrumentation

**DBS (Capillary) Blood**
- No phlebotomist necessary
- No venipuncture
- Less invasive and painless collection
- Collection of dried whole blood spot or plasma spot
- Volumes are between 20uL to 50uL/spot
- Transport easy once dried & stable
- Long term storage inexpensive
- DBS used in limited clinical applications
- New standardized collection guidelines needed
- Analyses methods need to be adapted to DBS and/or new method development & validation is necessary
Simple, Non-Invasive Capillary Blood Collection

SeventhSense BIOSYSTEMS

Approved

Whatman FTA DMPK-C
TAP Close-Up
Touch-Activated Phlebotomy (TAP)

1. The Tap100 is placed on a clean area of the upper arm. A medical adhesive around the base forms an airtight seal against the skin.

2. When the button is pushed, an array of microneedles creates temporary microincisions in the skin.

3. Blood enters the device and is collected in a reservoir which contains anticoagulants to preserve the sample.

4. An indicator signals when the blood collection is complete. The microincisions quickly close up after the device is removed.

Comparing alternatives (in microliters)

- Venipuncture (Blood taken with a needle from your vein): 4,000
- Seventh Sense TAP100: 100
- Standard Fingertick: 100
- Theranos “nanotainer” Fingertick: 25-50

www.usada.org
• Athletes rated TAP very favorably – quick and painless – 1-4 min
• TAP easy to operate by DCO
• Collection costs 1/10 of venipuncture
• Reliable and consistent volume collection
• Transfer from TAP to DBS requires special training
• Sample collection protocol completed
• Consistent size DBS are required for quantitative analyses
• Sample collection kit works well – based on current principles A&B
• Blood extraction manual but automation possible – lower costs
• Quantitative analysis of indirect biomarkers successful in cycling, marathon, weightlifting and mixed martial arts (MMA)
DBS Sample Collection Kits
DBS Field Experience – Blotting the DBS

130 mm
Spots of Unequal Volume:
Specialized Analytical Equipment
Tasso Blood Sampling Device
Excise 20 μL spot

Washing to remove soluble proteins
Red spot turns white

Cell membranes and membrane proteins remain on spot

Trypsin digest into peptides 2 hr

Quantify peptides by LC-MS/MS

Total Time: 4 hours

Comparison of DBS to Sysmex Values

Deming fit

DBS IRC/µl x 10

Sysmex IRC/µl x 10

Deming fit

DBS RBC/µl x 10^4

Sysmex RBC/µl x 10^4

Deming fit
Detection of Autologous Blood Transfusion

Study Outline:

- **BL1, BL2, BL3**: Baseline measurements
- **OUT**: Blood sample withdrawal
- **OUT + 2**: Blood sample collection
- **D0, D1, D2, D3, D4, D5, D6, D13, D20, D27, D34**: Days after blood collection

- **2-4 Weeks**: Period of blood withdrawal
- **21 Days**: Interval between blood withdrawal and collection

- **1 Unit Removed (all subjects)**: Blood sample withdrawal
- **1 Unit Transfused or Saline**: Blood transfusion or saline administration

- **Blood**: 15 subjects
- **Saline**: 11 subjects
Detection of Autologous Blood Transfusion

DBS IGF-1 Measurement

Dried Blood Spots (DBS) - Summary

• DBS is a promising complementary method to increase detection and deterrence; however urine and venous blood remain the gold standard
• DBS offer significant advantages over phlebotomy-derived blood samples
• Collection equipment and procedures, transport and analysis methods must be harmonized and standardized in order to be fit-for-purpose for routine doping control
• DBS may be an excellent alternative approach for specific athlete populations, introduction to doping control, and to inform target testing strategies
• TAP 2.0 refinements coming in the future – 250uL volume, EDTA
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