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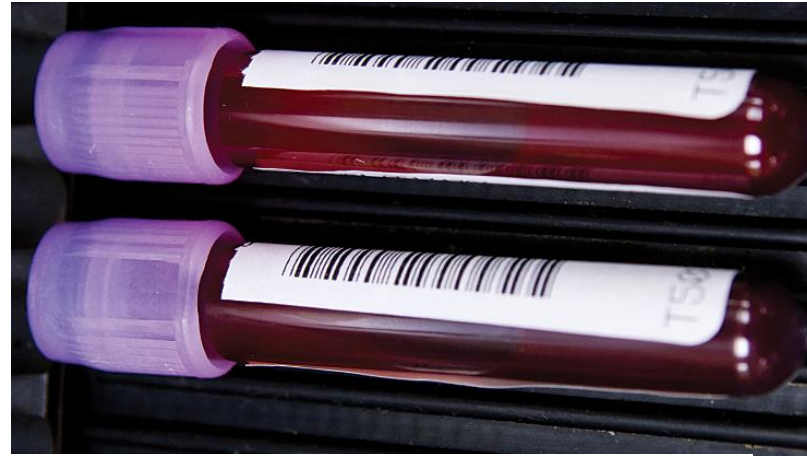
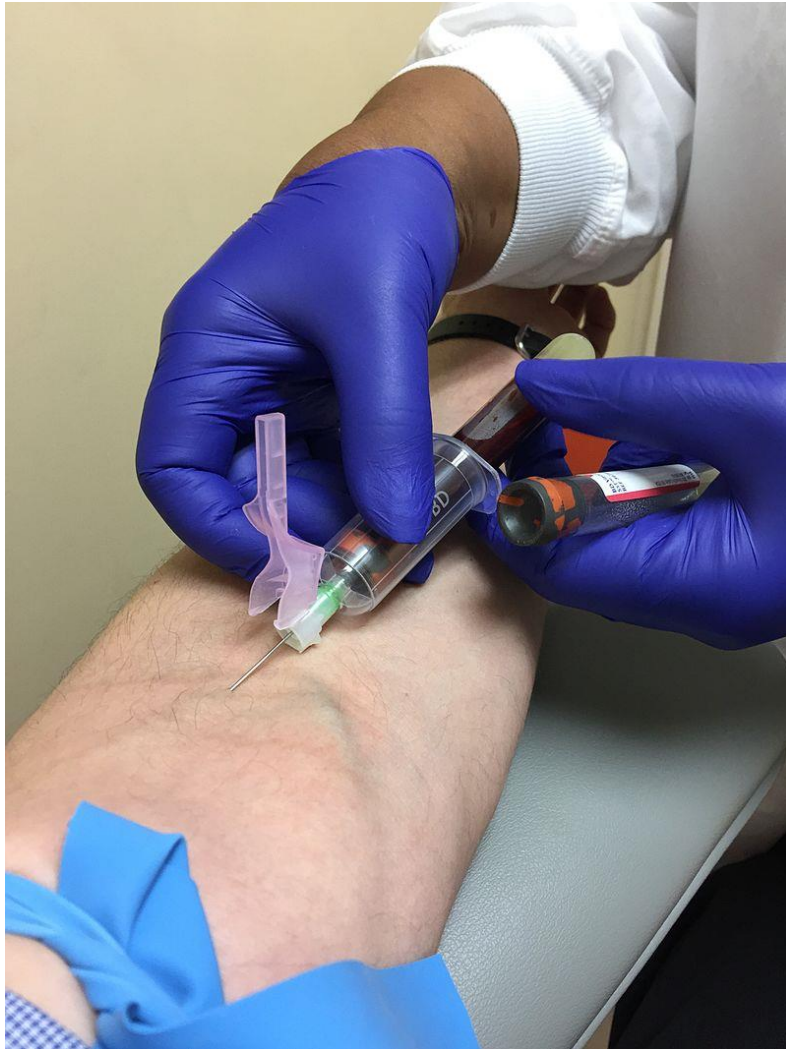
**U.S. Anti-Doping Agency**

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

*WADA ABP Symposium 2018*

*Matthew Fedoruk, PhD*

# The Typical Athlete Experience



# Current Blood Testing Strategies



WHOLE BLOOD



SERUM



Complete Blood  
Count  
(ABP parameters)

ESA Testing

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



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# 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

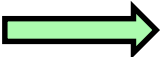
- No phlebotomist necessary
- No venipuncture
- Less invasive and painless collection
- Collection of dried whole blood spot or plasma spot
- Volumes are between 20uL to 50uL/spot
- Transport easy once dried & stable
- Long term storage inexpensive
- DBS used in limited clinical applications
- New standardized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



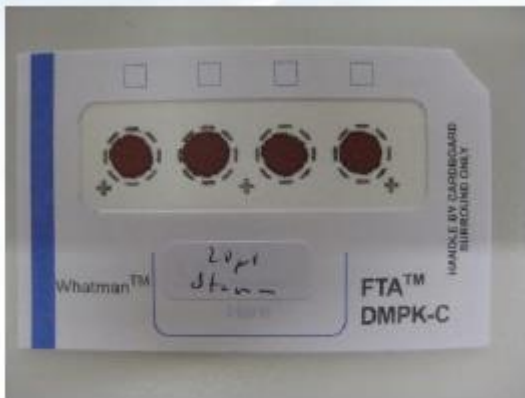
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# Simple, Non-Invasive Capillary Blood Collection

CE FDA  
Approved



SeventhSense  
BIOSYSTEMS



Whatman FTA DMPK-C

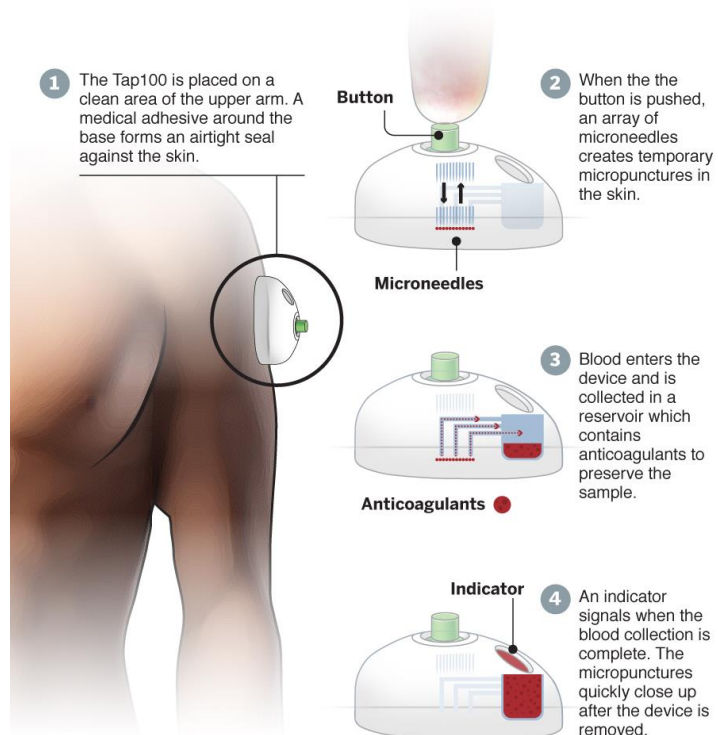


# TAP Close-Up





# Touch-Activated Phlebotomy (TAP)



**4,000**  
(Average per vial)

Venipuncture  
(Blood taken with a needle from your vein)

### Comparing alternatives (IN MICROLITERS)

**100**  
Seventh Sense TAP100

**100**  
Standard fingerstick

**25-50**  
Theranos "nanotainer" fingerstick



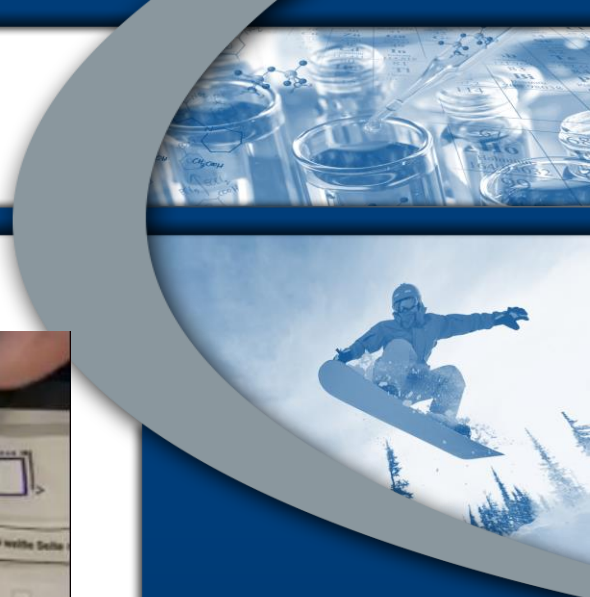
# DBS Collection

- Athletes rated TAP very favorably – quick and painless – 1-4min
- 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)

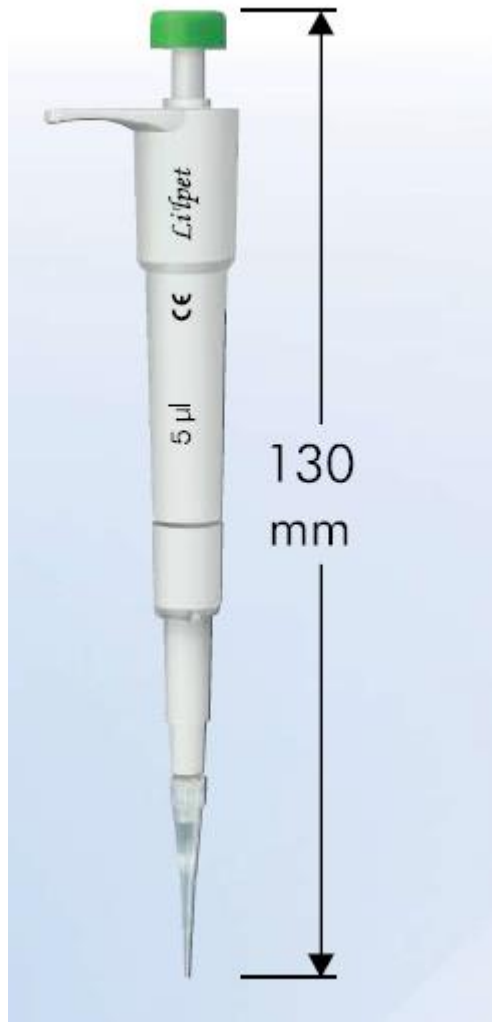


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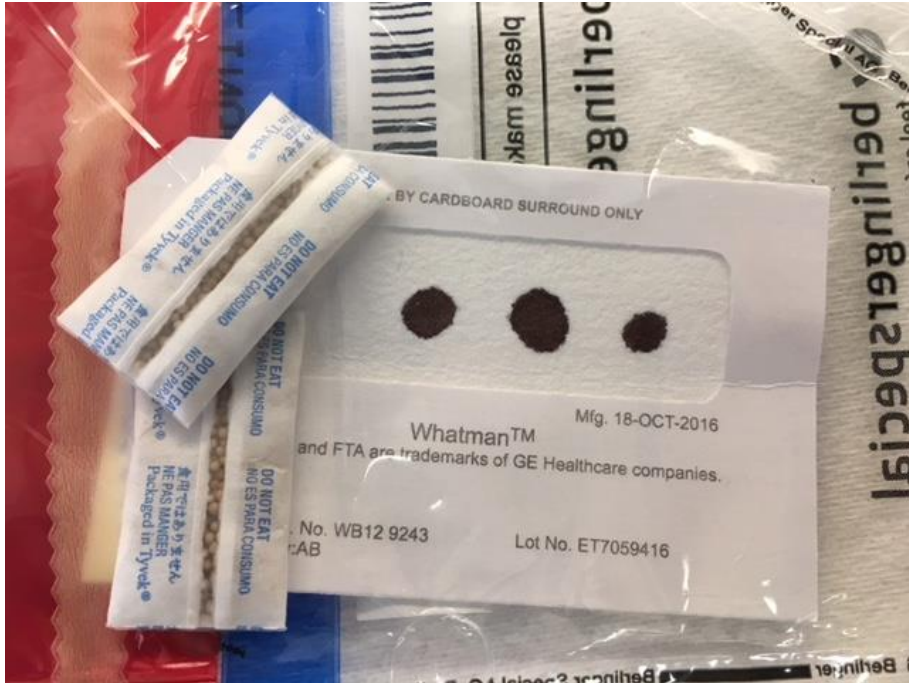
# DBS Sample Collection Kits



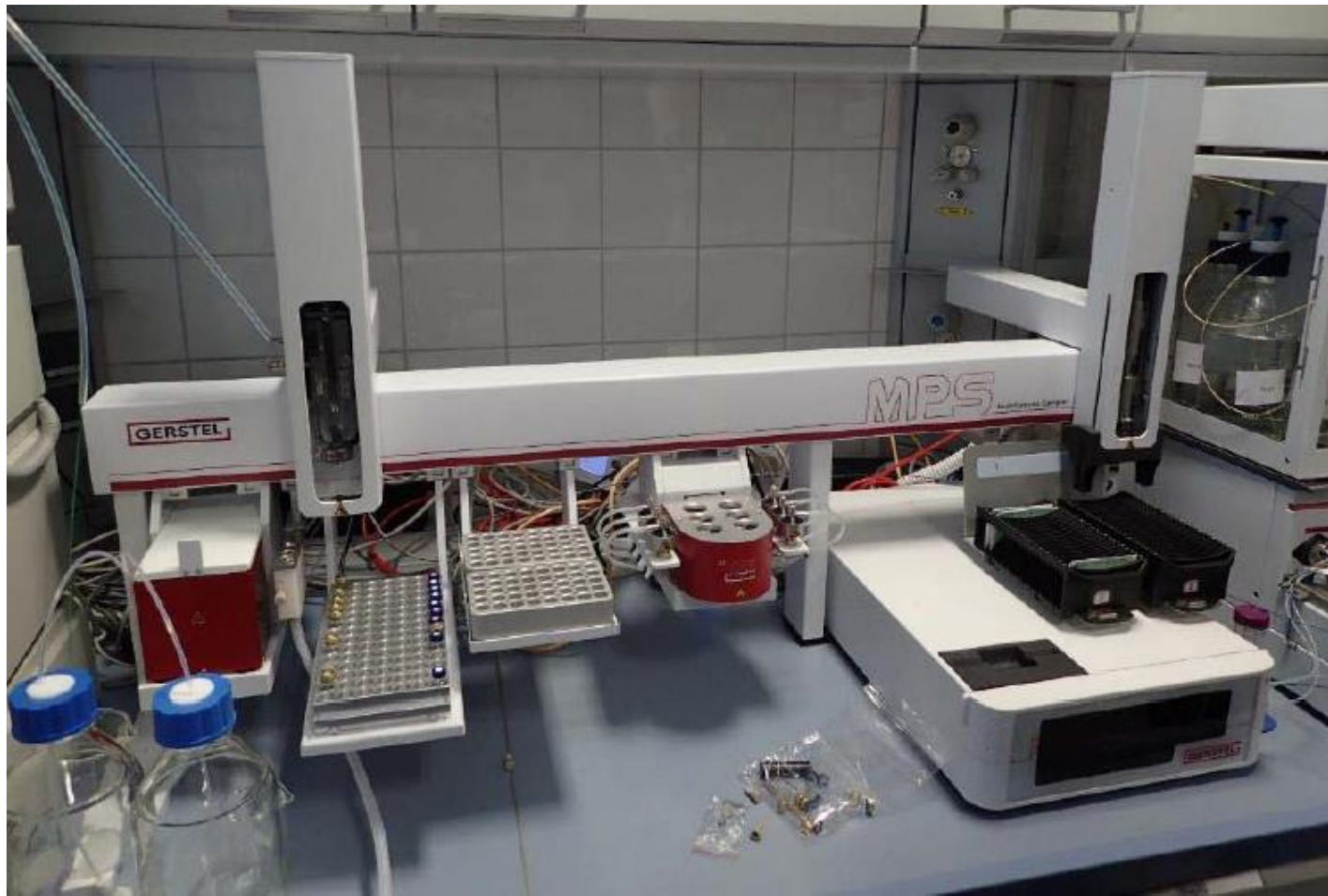
# DBS Field Experience – Blotting the DBS



# Spots of Unequal Volume:



# Specialized Analytical Equipment



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# Tasso Blood Sampling Device



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# DBS Indirect ABP Parameters Method Outline

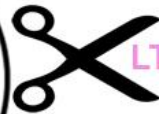
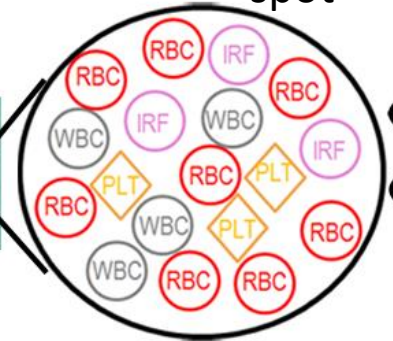
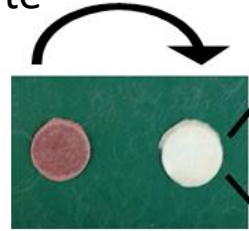
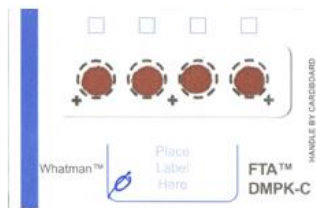
Excise 20  $\mu$ L spot

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

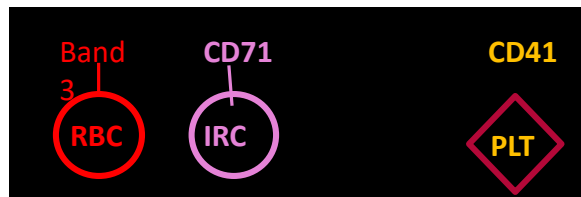
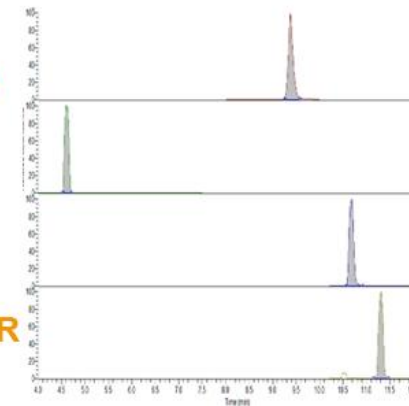


IPPDSEATLVLVGR

LTDFGNAEK

LFLAEFQSIPR

GQVLVFLGQSEGLR



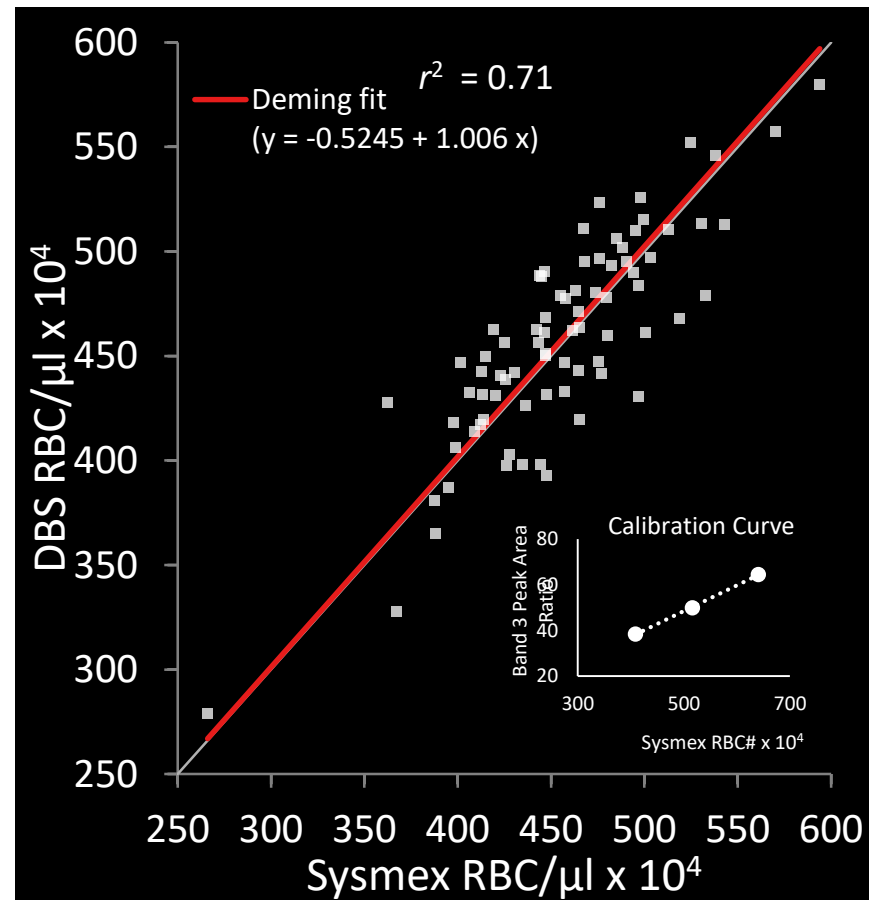
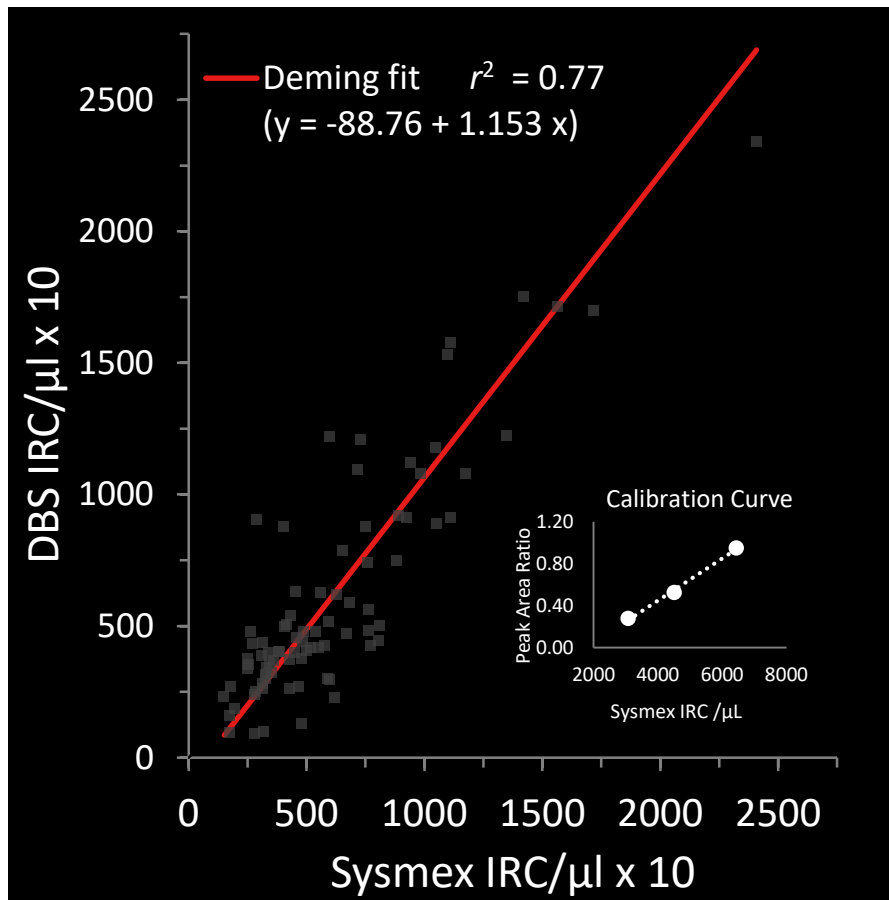
Total Time: 4 hours

H.D. Cox, D. Eichner. A mass spectrometry method to measure membrane proteins in dried blood spots for the detection of blood doping practices in sport. *Analytical Chemistry* (2017) 89(18) 10029.

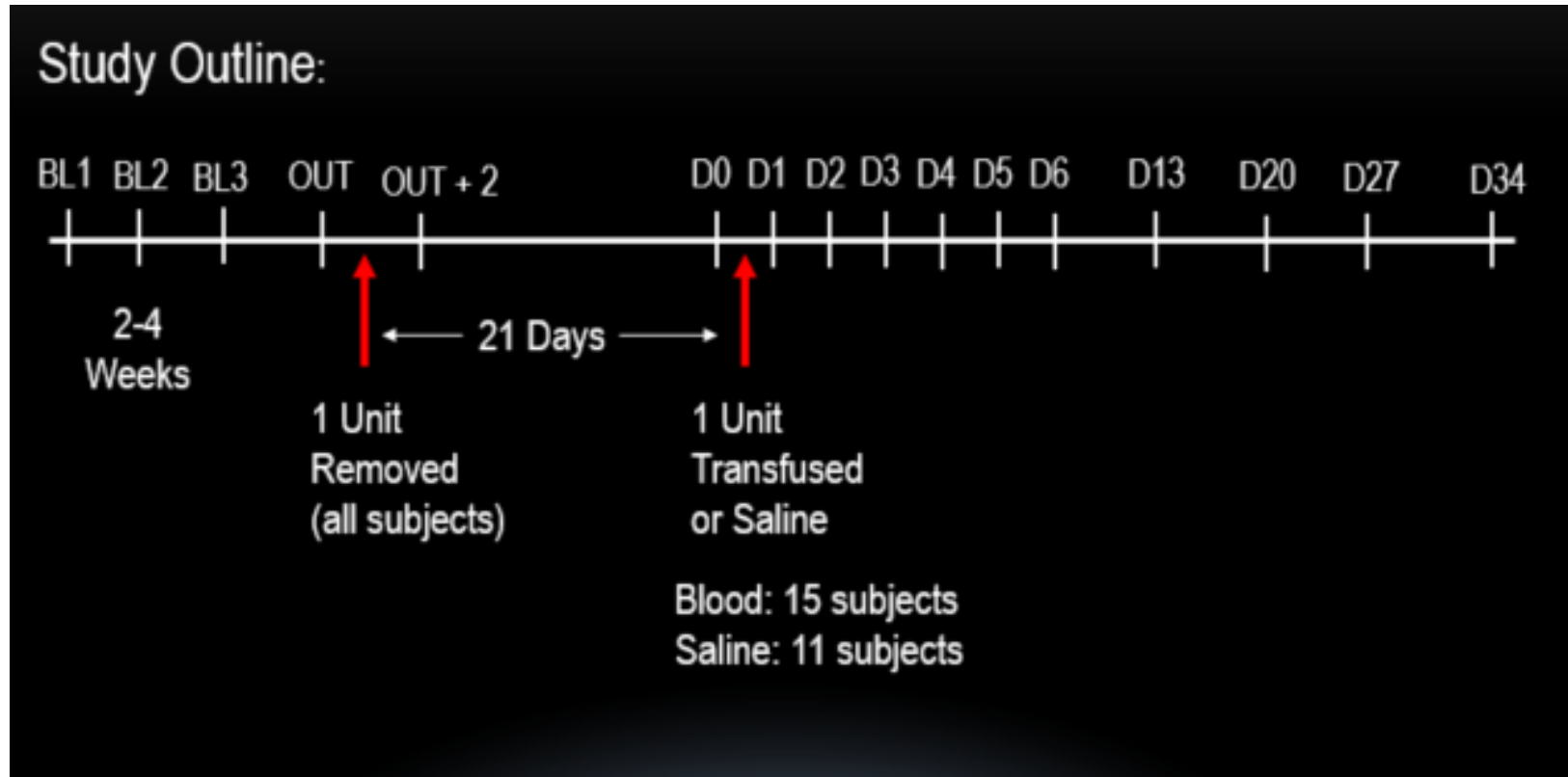




# Comparison of DBS to Sysmex Values



# Detection of Autologous Blood Transfusion

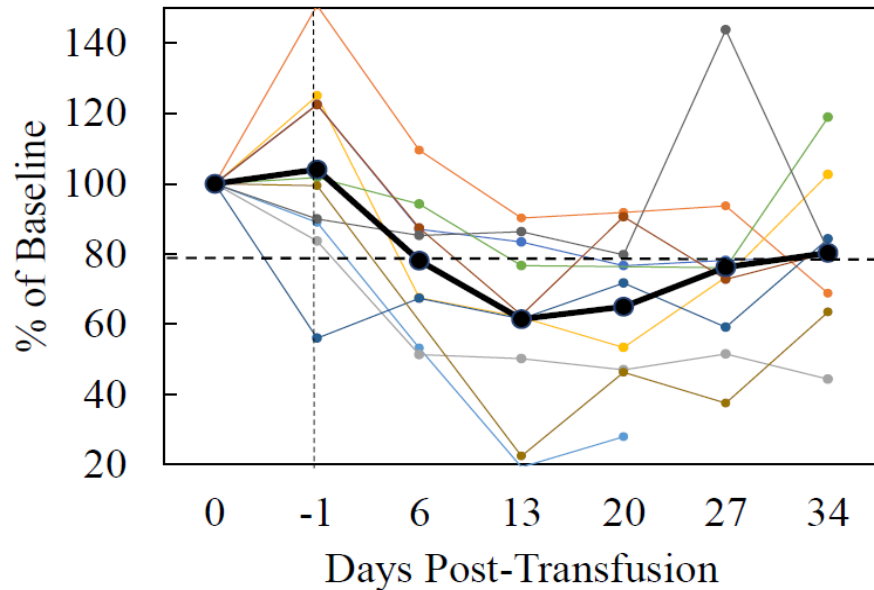


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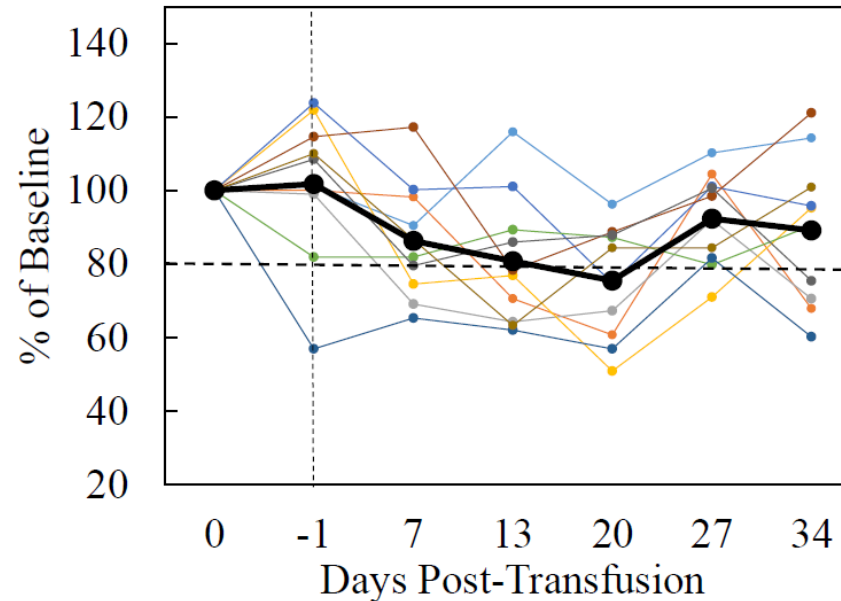
# Detection of Autologous Blood Transfusion



### DBS CD71/Band3 Ratio



### Reticuloctye% - Current

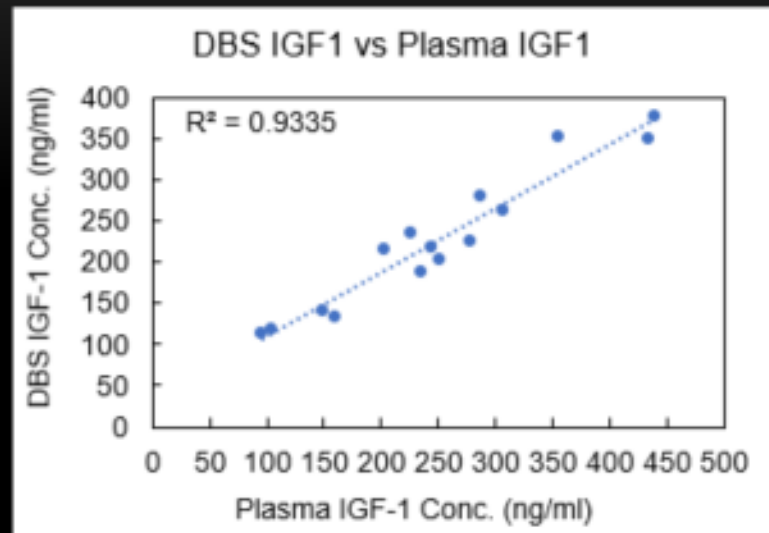
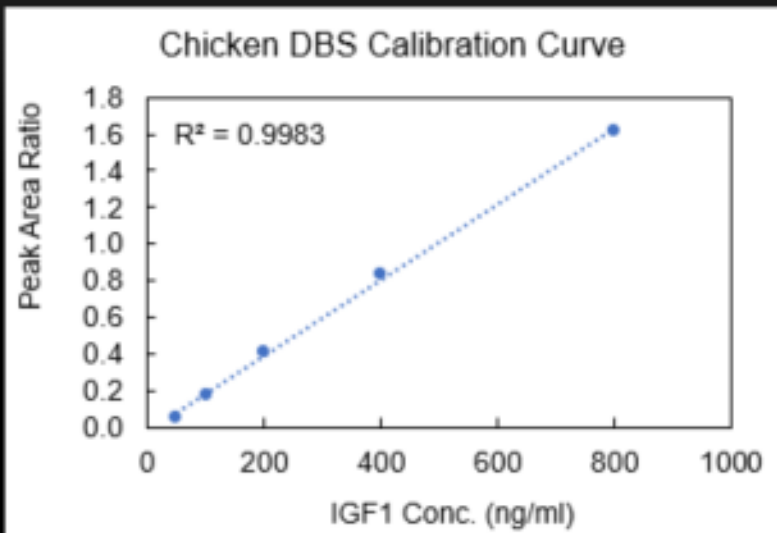


HD Cox et. al. Detection of autologous blood transfusion using a novel dried blood spot method. *Drug Testing and Analysis* (2017) 9(11-12) 1713-1720.



# DBS IGF-1 Measurement

## Top-Down Method



1093 > 473      y5, 1+  
1197            b65, 6+  
1068            b68, 7+  
1246            b68, 6+  
1436

LLOQ: 50 ng/ml  
AVE %BIAS: -6.7%  
3-Day %CV QC1: 4.7%  
                  QC2: 4.8%

IGF-1 Method Based on previous work: Cox, HD et. al. *Anal Bioanal. Chem.* (2013) 405(6) 1949.

# 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

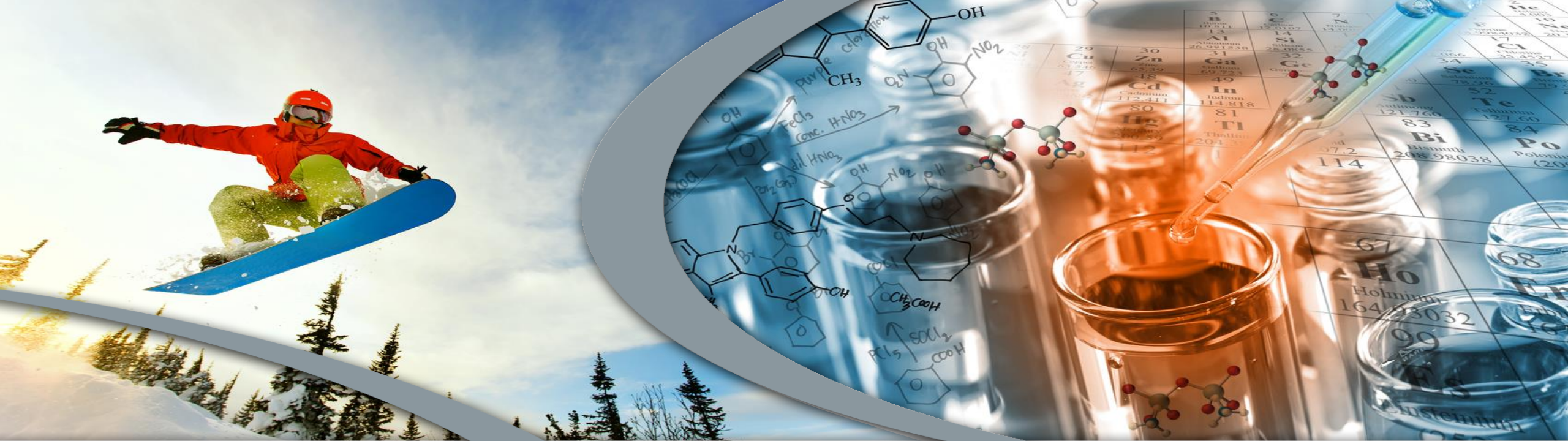


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# Acknowledgements

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- SMRTL – Dr. Daniel Eichner, Dr. Geoff Miller, Dr. Holly Cox and technical staff
- Seventh Sense Biosystems
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- Berlinger
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- Dr. Oliver Rabin & Dr. Neil Robinson





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**Thank you!**

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