

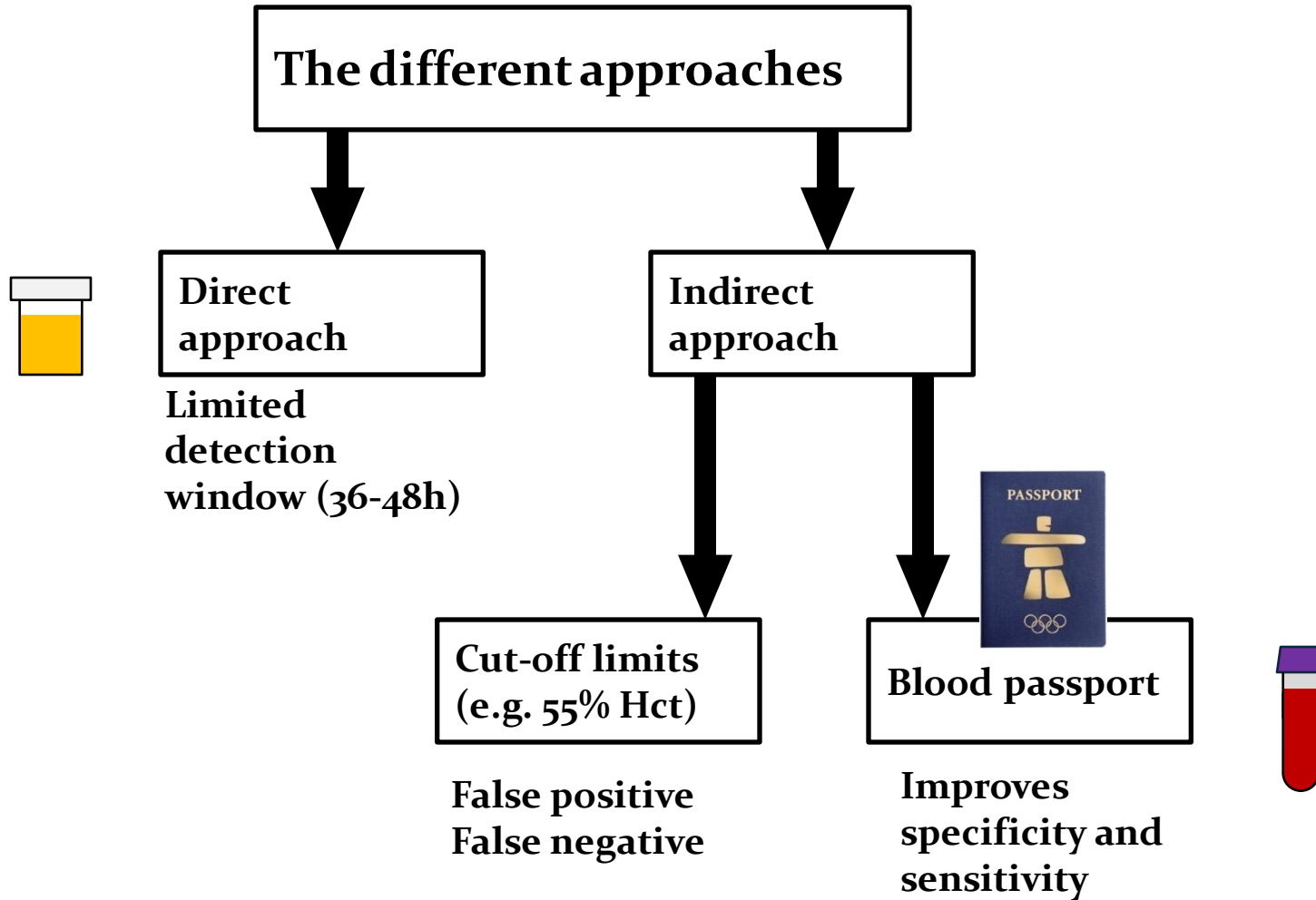
# Genetic Signatures: Application to EPO detection

**Dr Yannis Pitsiladis, on behalf of the Glasgow "OMICS" consortium**

College of Medicine, Veterinary and Life Sciences  
Institute of Cardiovascular and Medical Sciences  
University of Glasgow  
Glasgow, Scotland



# Epo anti-doping tests



# The Problem



*Despite more than 250,000 tests being carried out in the last year [2010], only 36 came back as positive for EPO.*

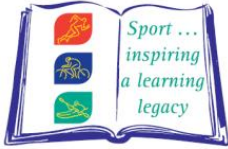
*“We are catching the dopey dopers, but not the sophisticated ones”.*



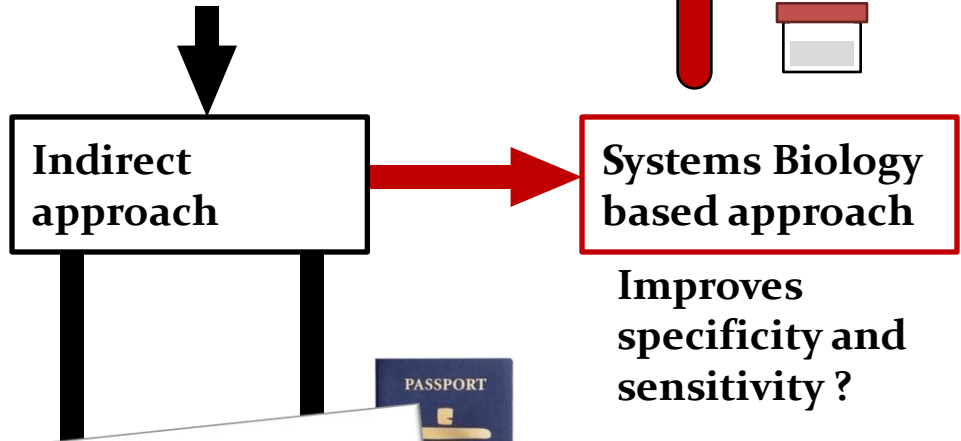
David Howman  
director general of WADA

# Epo anti-doping tests

**ICSEMIS 2012**



International Convention on Science,  
Education & Medicine in Sport  
Scottish Exhibition & Conference Centre, Glasgow, UK  
19-24 July 2012

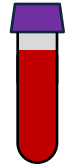


ORIGINAL ARTICLE

**Current markers of the Athlete Blood Passport do not flag microdose EPO doping**

Michael Ashenden · Clare E. Gough · Andrew Garnham · Christopher J. Gore · Ken Sharpe

Received: 23 November 2010 / Accepted: 3 February 2011  
© Springer-Verlag 2011



# The Omics Cascade ≠ Systems Biology

What CAN happen

GENOMICS

BIOINFORMATICS

What APPEARS  
to happen

TRANSCRIPTOMICS

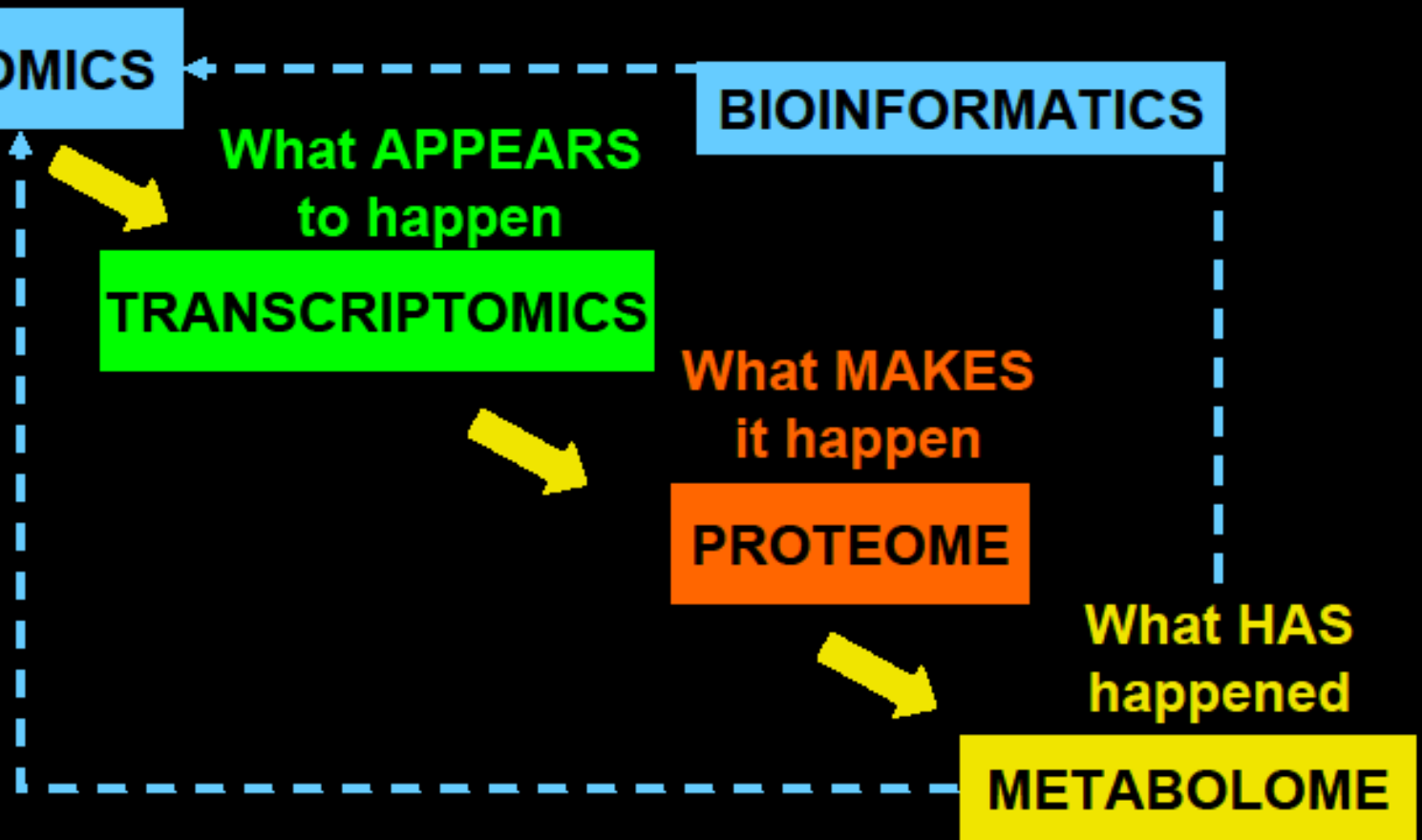
What MAKES  
it happen

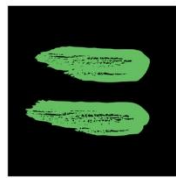
PROTEOME

What HAS  
happened

METABOLOME

PHENOTYPE





**WORLD  
ANTI-DOPING  
AGENCY**

play true

## **World Anti-Doping Agency (WADA) Health, Medical, and Research Committee**

### **Application Form for Scientific Research Grants – 2008 Competition**

**Date:**

**Project category:**

- Compounds and/or methods enhancing oxygen delivery
- Non-steroidal compounds or methods enhancing growth
- Projects relating to the Prohibited List: classical methodologies
- Projects relating to the Prohibited List: novel methodologies
- Identification and/or detection of substances with suspected doping potential

**Gmeiner Günter**, Head of WADA-accredited Doping Control Laboratory, Austrian Research Centres GmbH – ARC, Doping Control Laboratory, A-2444, Seibersdorf, Austria

# Research hypotheses and objectives

1. To measure blood parameters and gene-expression profiles in sea-level and altitude-adapted trained athletes after r-HuEpo administration;
2. Determine the effects of ethnicity on haematological parameters and gene-expression profiles; and
3. Formulate revised methods with improved discriminatory power relative to standard haemoglobin and haematocrit detection protocols.



**WORLD  
ANTI-DOPING  
AGENCY**

play true

Cohort	No of samples
Epo trial east African runners (n=20) x 19 serial blood samples	380
Epo trial Caucasian runners (n=20) x 19 serial blood samples	380
Total samples possible	760



# Study design

Endurance trained (Caucasian, sea level, n = 20)

Endurance trained (East Africans, altitude, n = 20)



↓ = sample collection

↑ = rHuEpo injection  
(50 IU/kg)



RNA from whole blood



Whole blood/Plasma



Serum



RNA from saliva



Urine

## Demographic characteristics of elite Kenyan endurance runners

VINCENT O. ONYWERA<sup>1</sup>, ROBERT A. SCOTT<sup>2</sup>, MICHAEL K. BOIT<sup>1</sup>, &  
YANNIS P. PITSIKALIS<sup>1,2</sup>

<sup>1</sup>Department of Exercise and Sports Science, Kenyatta University, Nairobi, Kenya, and <sup>2</sup>International Centre for East African Running Science, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK

# KENYAN REGIONS

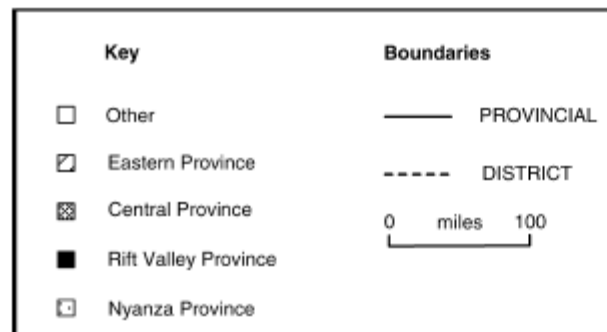
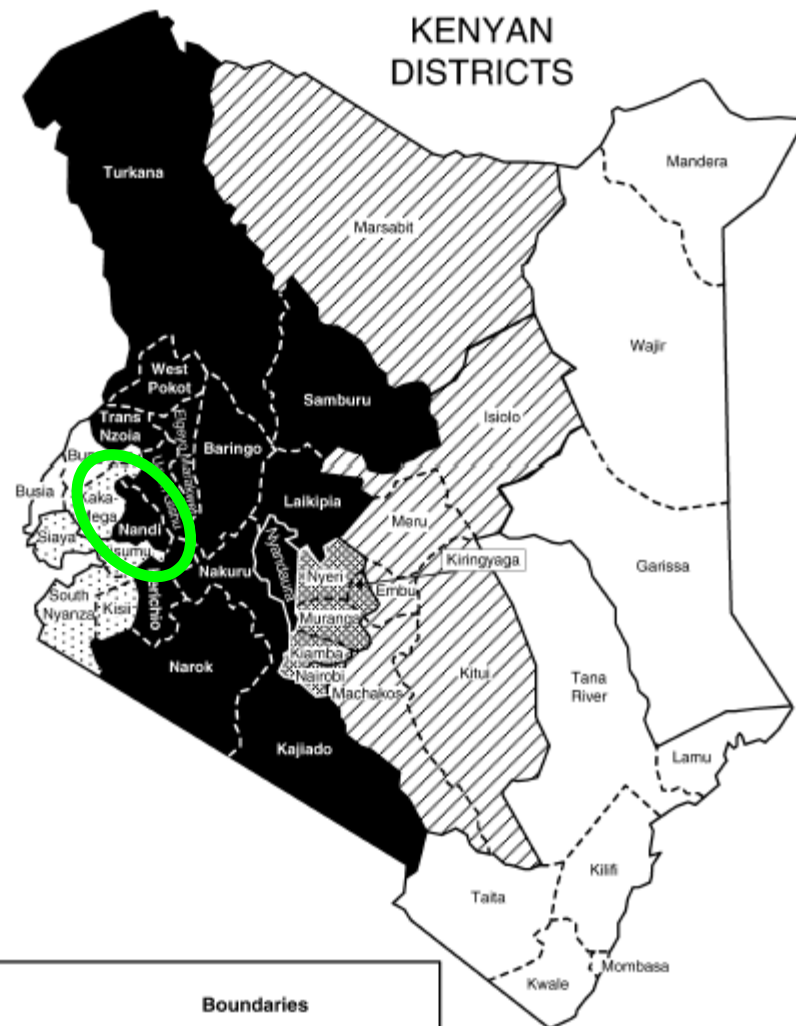
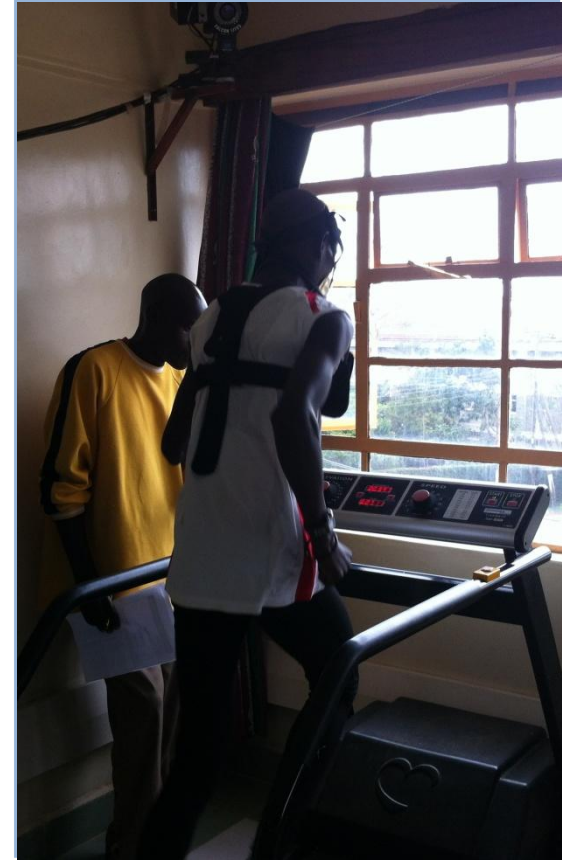


Figure 1. Districts of Kenya reflecting distribution of participants in the present study. "Other" covers Coast, North-Eastern and Western provinces.

# Maximum aerobic capacity ( $\text{VO}_2\text{max}$ )

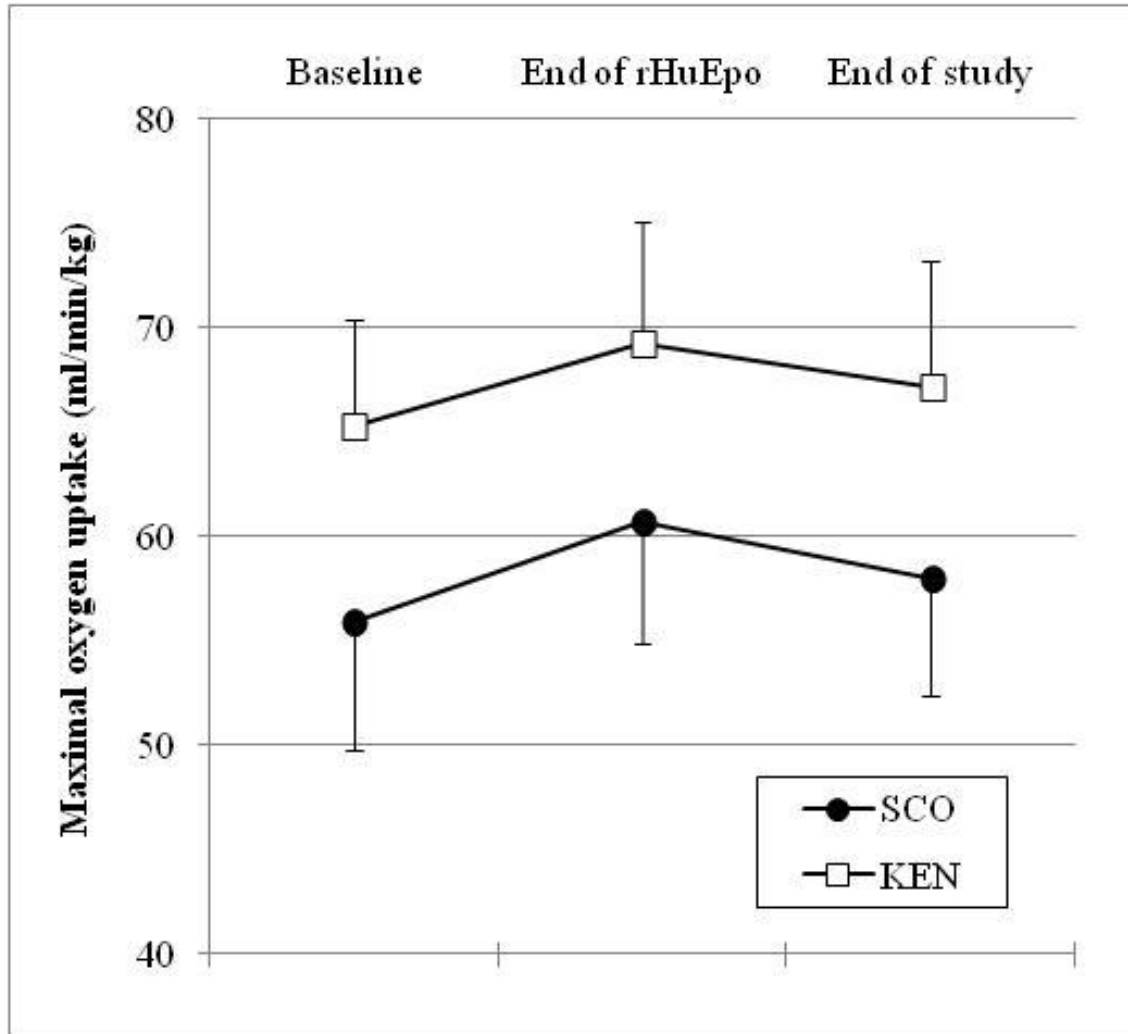


WADA supported Human Performance Lab, Glasgow, Scotland.

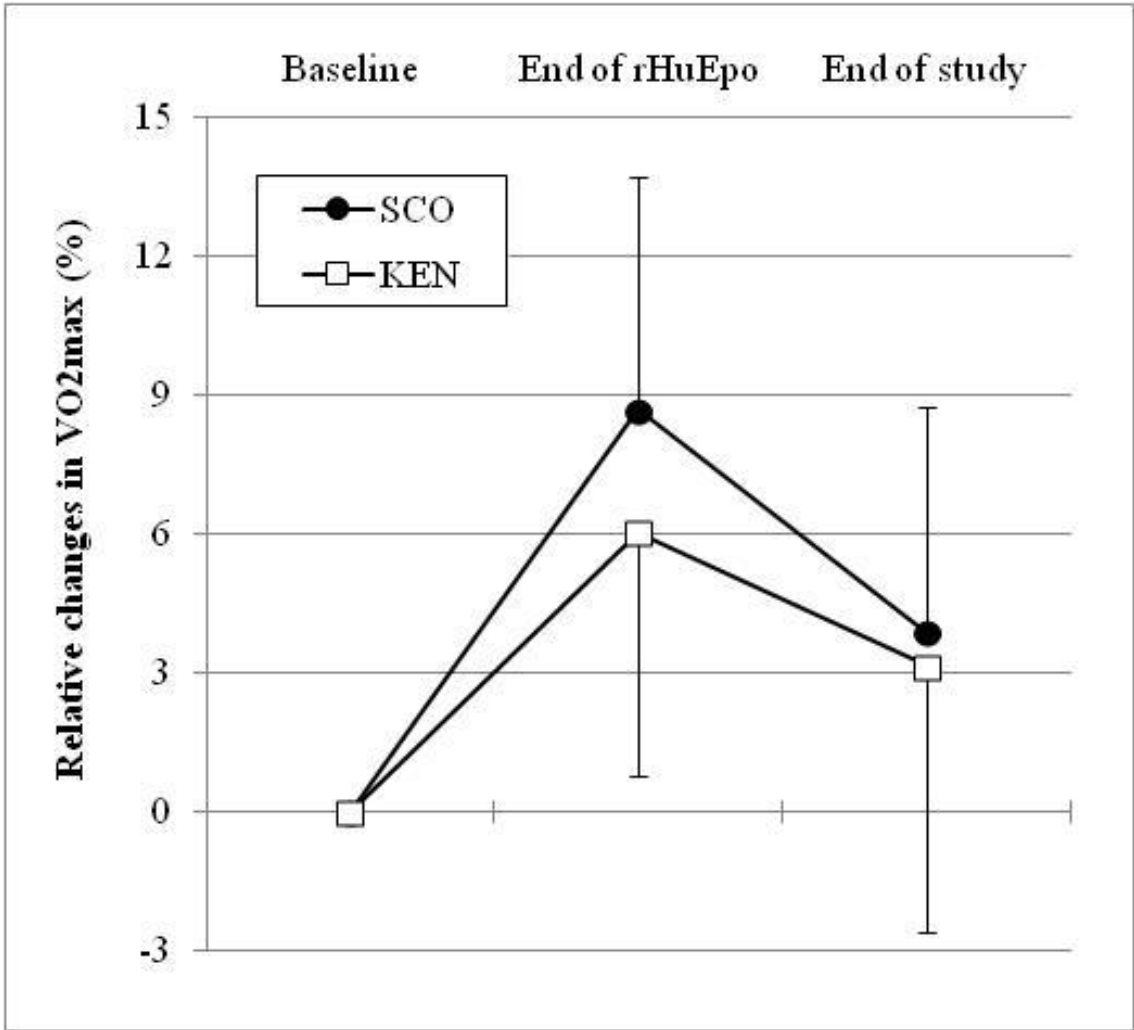


WADA supported Human Performance Lab, Eldoret, Kenya.

VO<sub>2</sub>max



VO<sub>2</sub>max





Indoor athletic track (200 m) at Kelvin Hall International Sports Arena, Glasgow, Scotland.

## Erythropoietin doping in cycling: lack of evidence for efficacy and a negative risk–benefit

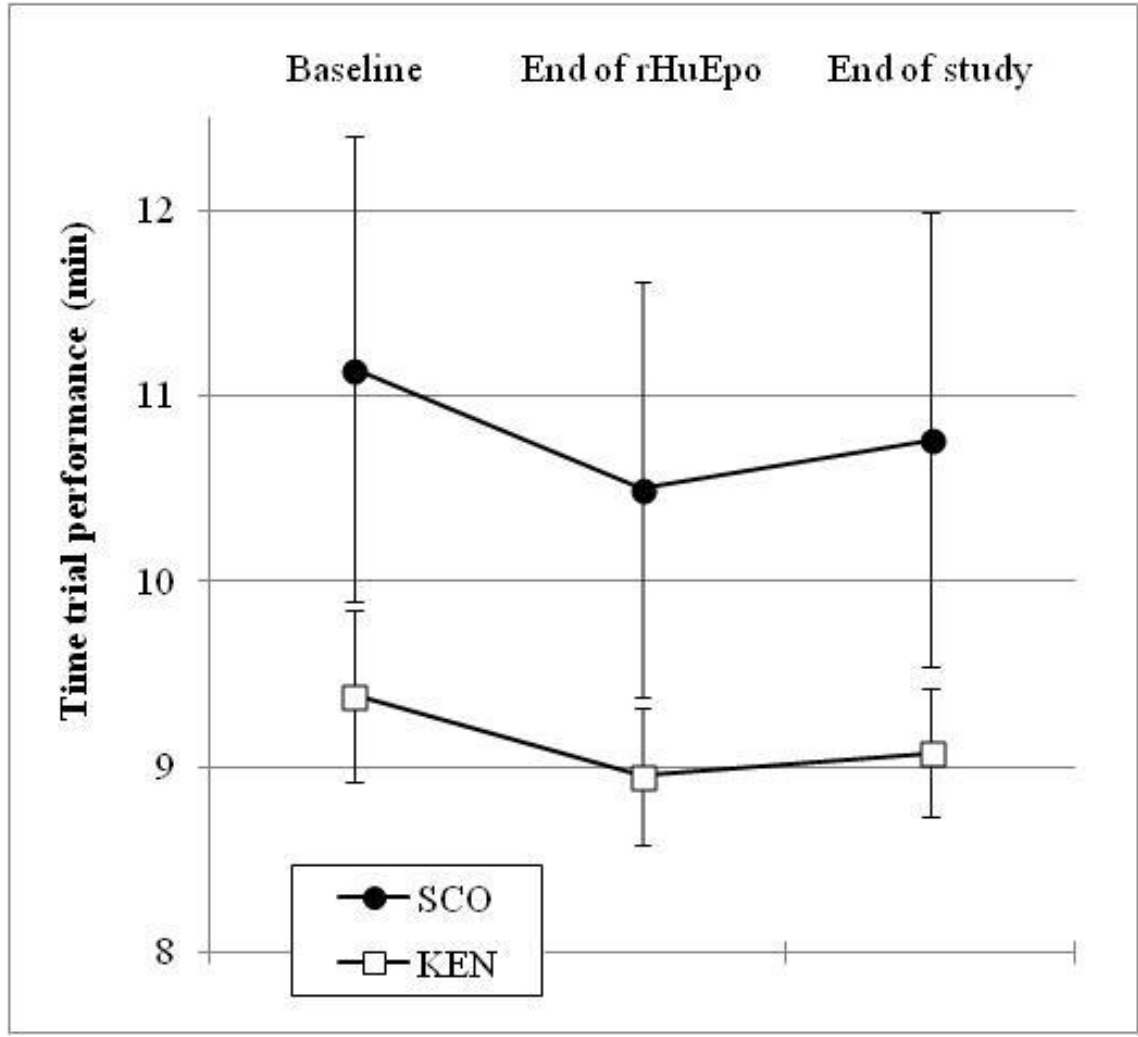
Jules A. A. C. Heuberger,<sup>1</sup> Joost M. Cohen Tervaert,<sup>1</sup> Femke M. L. Schepers,<sup>1</sup> Adriaan D. B. Vliegenthart,<sup>1</sup> Joris I. Rotmans,<sup>2</sup> Johannes M. A. Daniels,<sup>3</sup> Jacobus Burggraaf<sup>4</sup> & Adam F. Cohen<sup>5</sup>

<sup>1</sup>Biopharmaceutical Sciences, Leiden University, Leiden, <sup>2</sup>Department of Nephrology, Leiden University Medical Centre, Leiden, <sup>3</sup>Department of Pulmonary Diseases, VU University Medical Centre, Amsterdam, <sup>4</sup>Leiden Amsterdam Centre for Drug Research, Leiden and <sup>5</sup>Leiden University Medical Centre, Leiden, The Netherlands

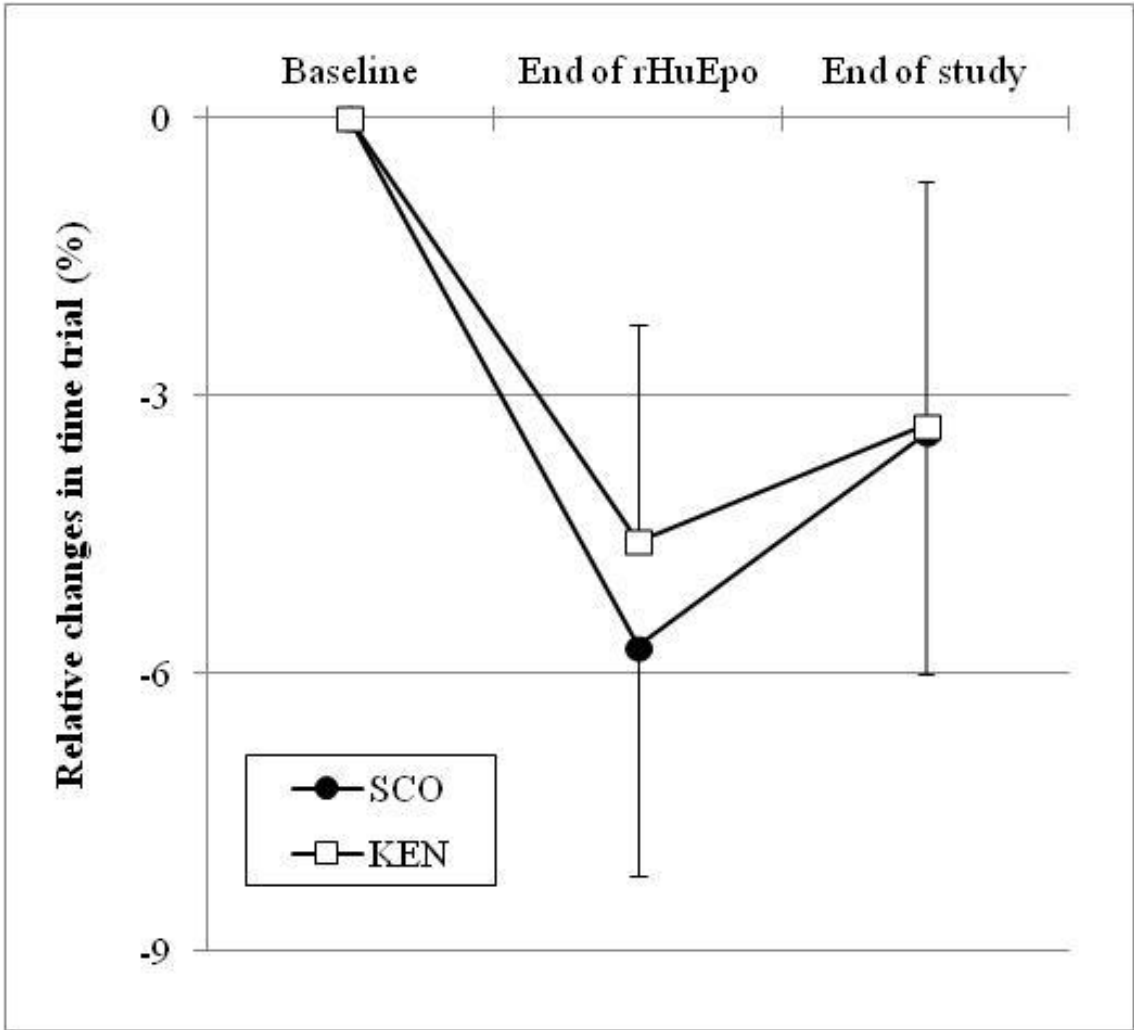


Outdoor athletic track (400 m) at Eldoret, Kenya.

# Time trial



Time trial





# Standard methods of analysis



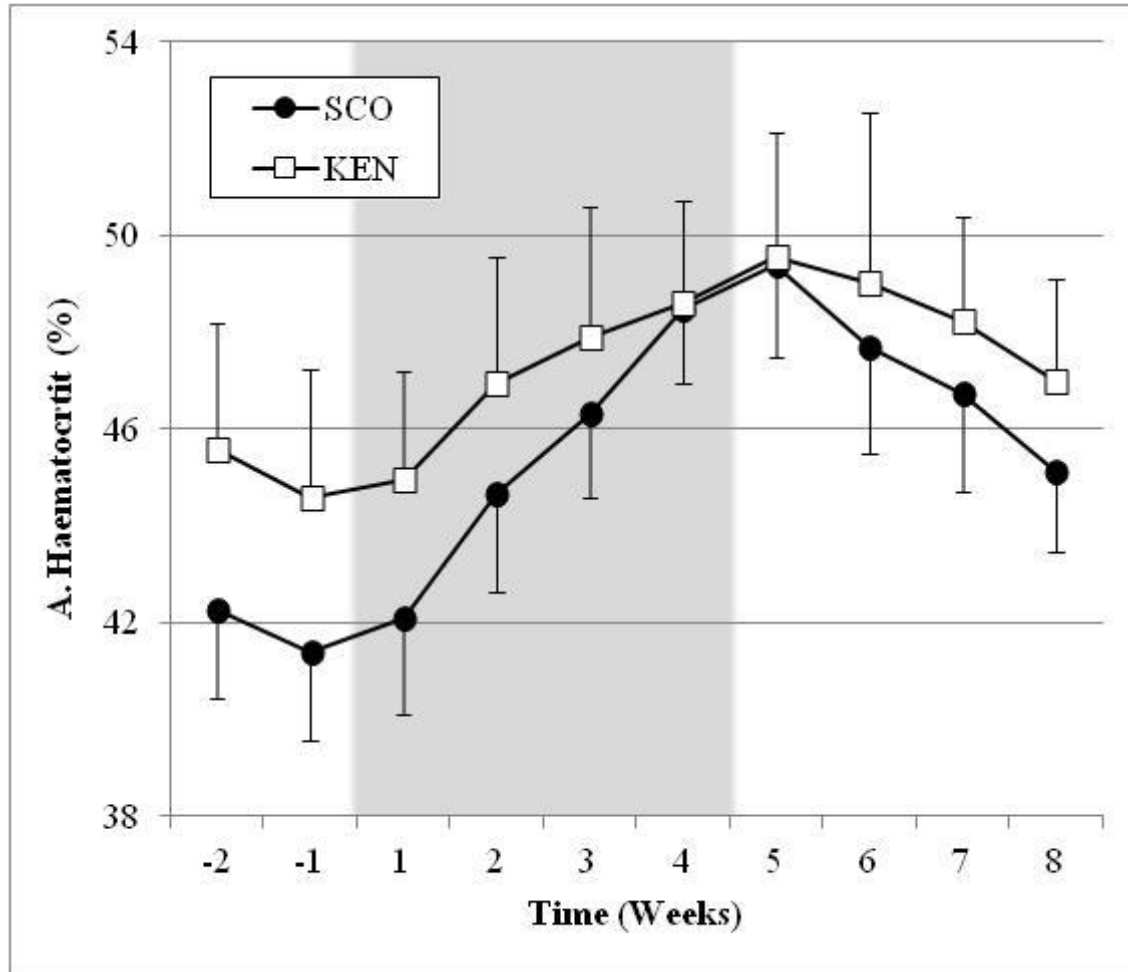
Sysmex XT-2000i

**SYSMEX UK**

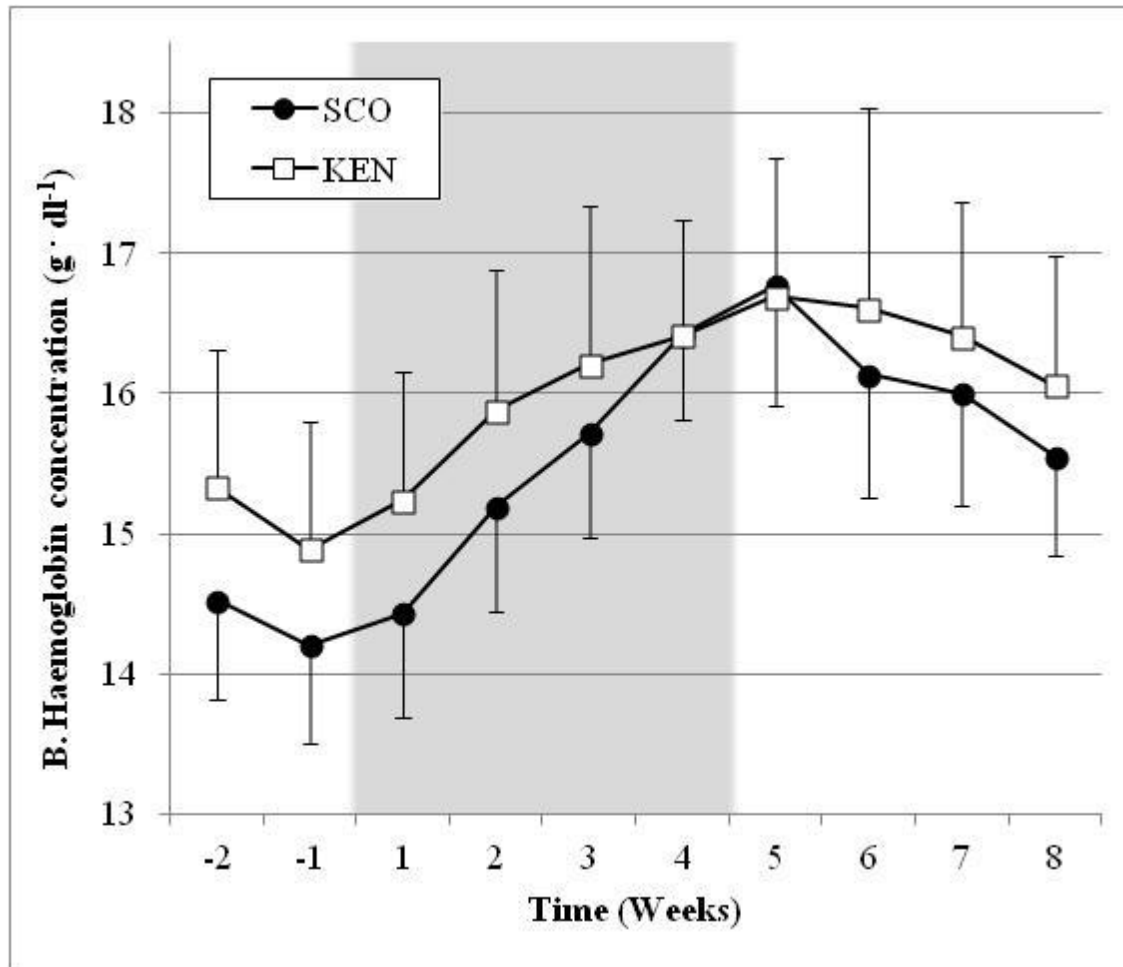
SOLUTIONS FOR LABORATORY SCIENTISTS  
FROM LABORATORY SCIENTISTS



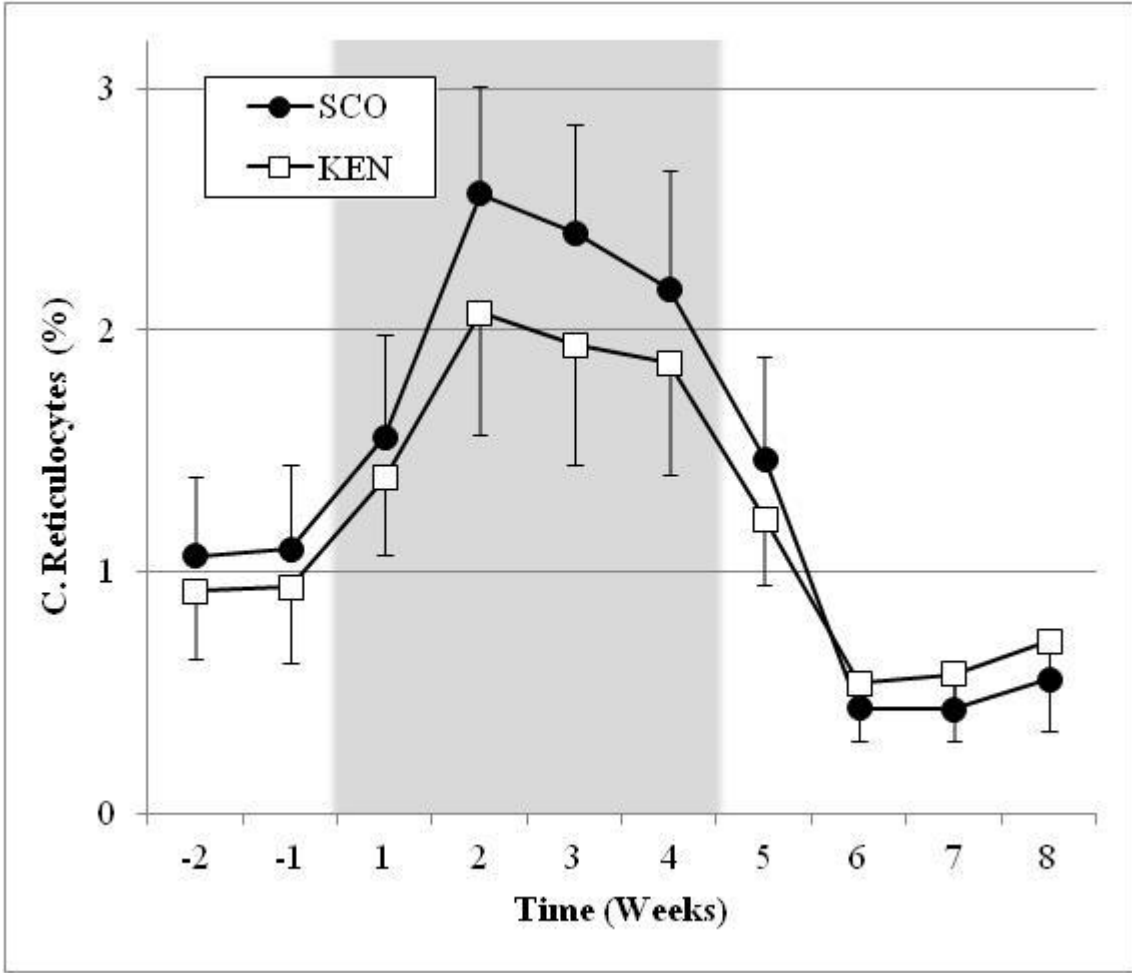
# Haematocrit



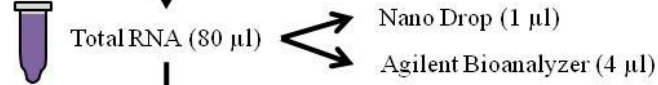
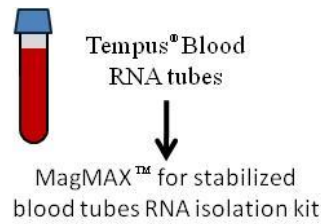
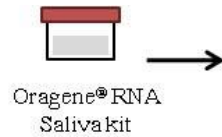
# Haemoglobin concentration



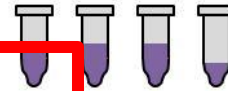
# Reticulocyte



# "Omics" workflow



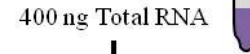
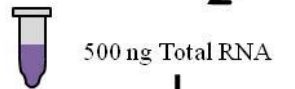
Total RNA aliquots (3 x 20 µl + remaining)



stored at -80°C

Gene expression microarray

miRNA expression profiling



Illumina<sup>®</sup> Total Prep<sup>™</sup> Reverse Transcription

Megaplex<sup>™</sup> Reverse Transcription



Illumina<sup>®</sup> Total Prep<sup>™</sup> in vitro Transcription

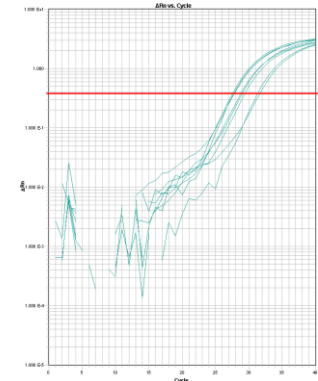
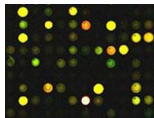
Quantitative real time PCR



Nano Drop  
Agilent Bioanalyzer

Hybridization

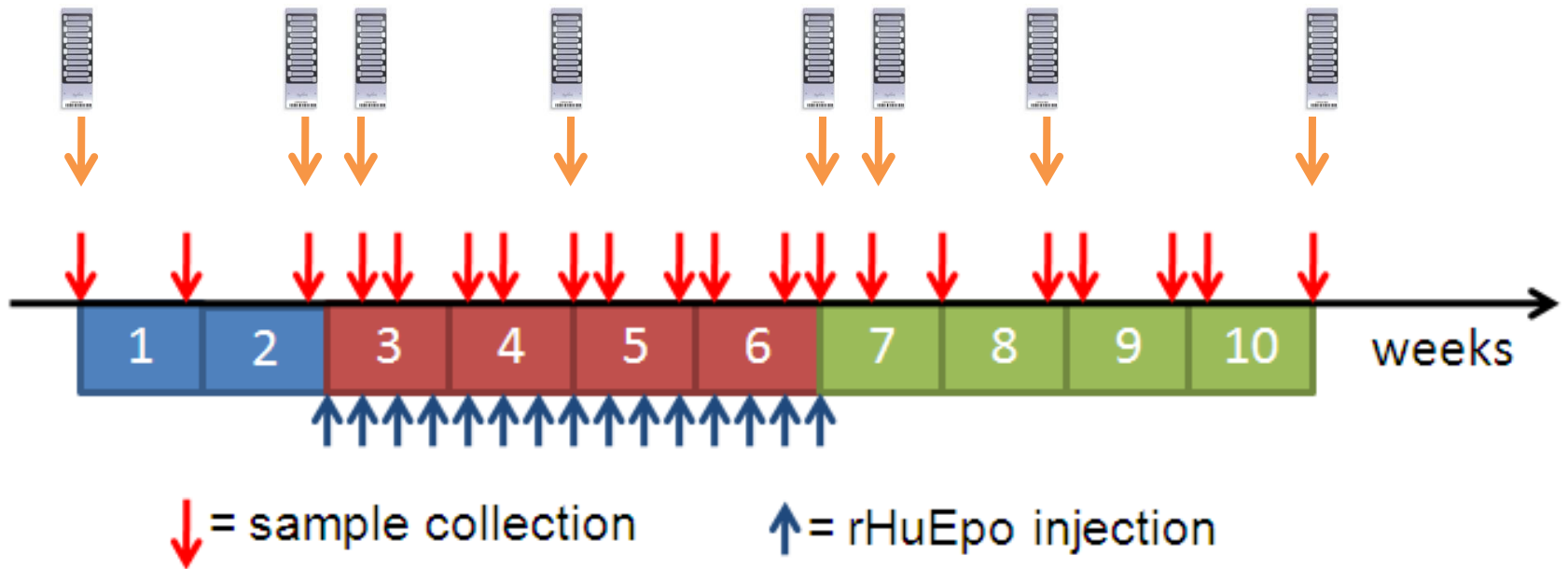
Illumina<sup>®</sup> BeadArray Reader



# Microarray experiment

8/20 time points:

2 Baseline, 3 during and 3 post r-HuEpo



5 % FDR significant and  $\geq 1.5FC$ : During rHuEpo

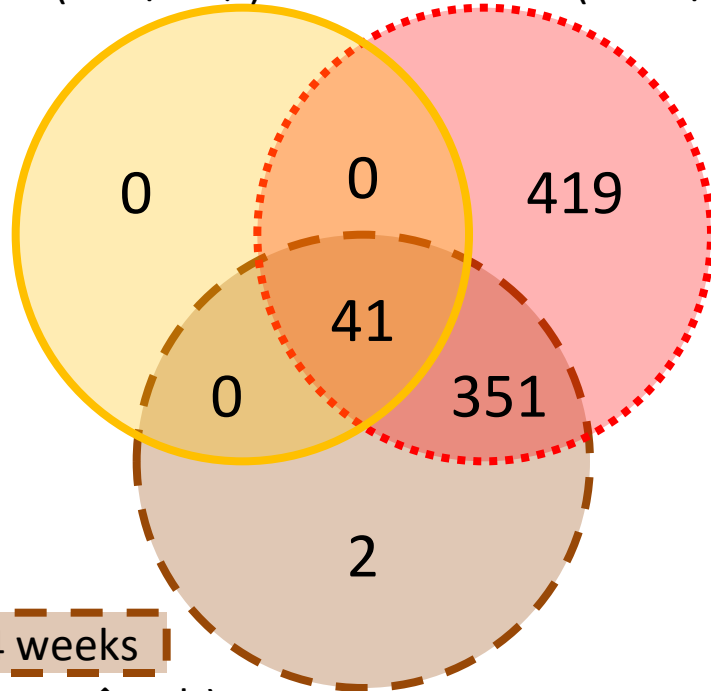
SCO (n=18)

2 days

41 (41  $\uparrow$  0 $\downarrow$ )

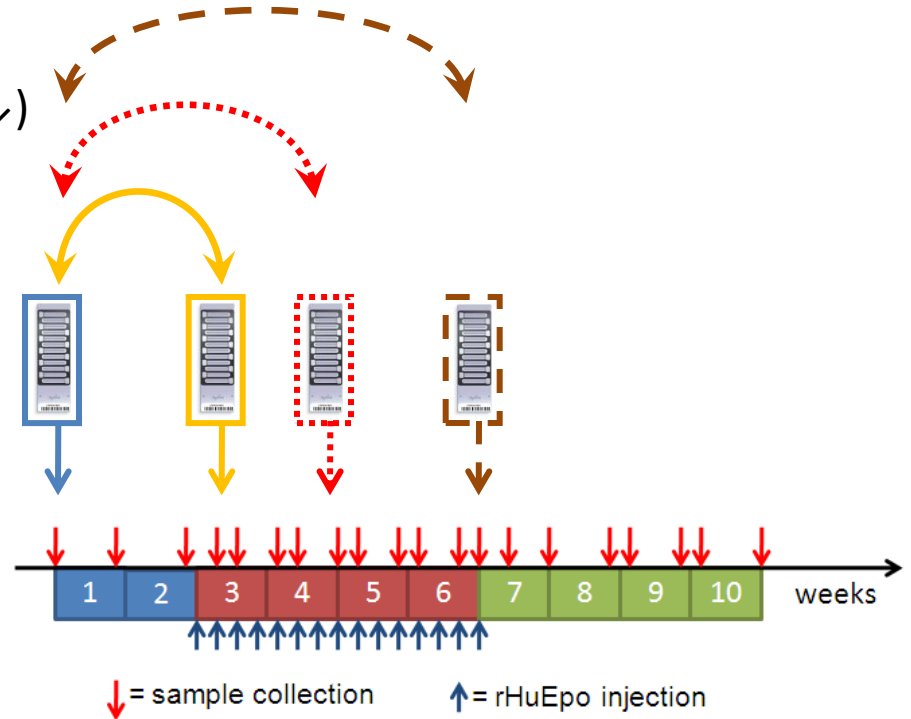
2 weeks

811 (736  $\uparrow$  75 $\downarrow$ )



4 weeks

394 (390  $\uparrow$  4 $\downarrow$ )



5 % FDR significant and  $\geq 1.5$ FC: During rHuEpo

SCO (n=18)

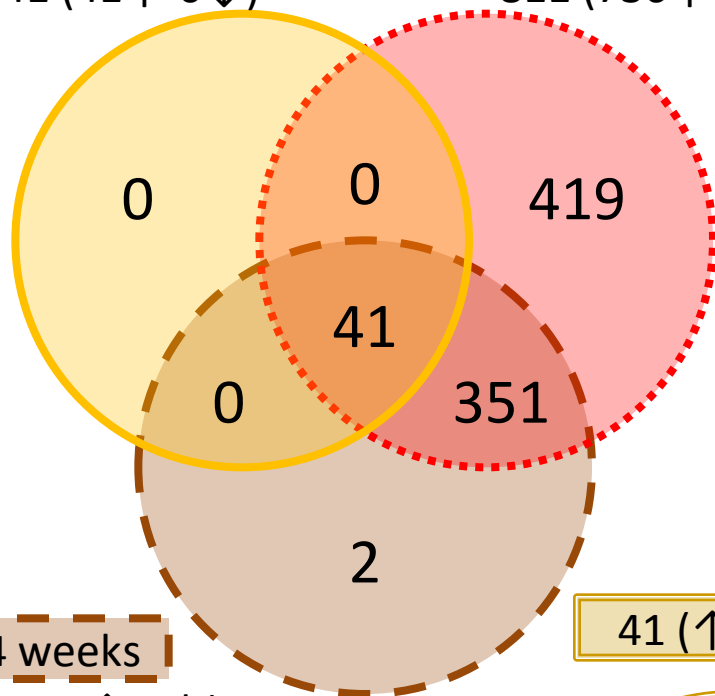
KEN (n=18) (n=6)

2 days

41 (41 $\uparrow$  0 $\downarrow$ )

2 weeks

811 (736 $\uparrow$  75 $\downarrow$ )

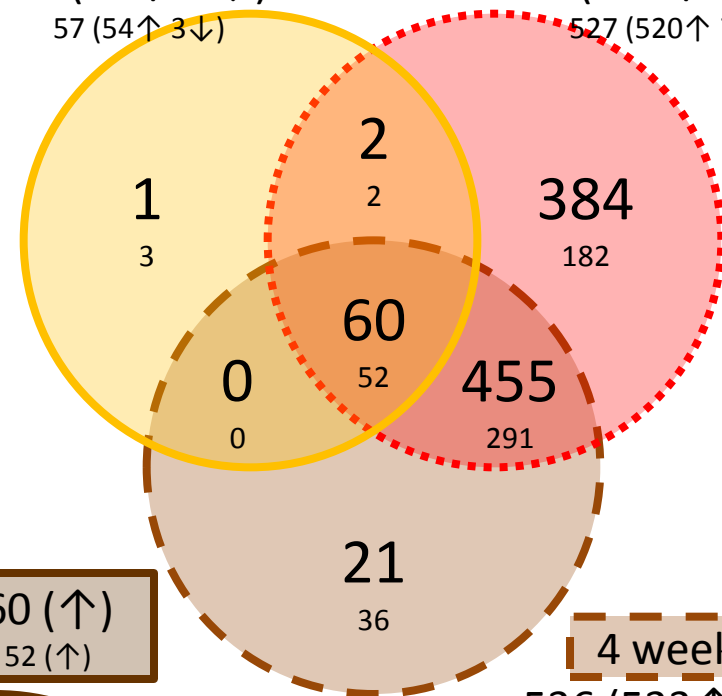


2 days

63 (63 $\uparrow$  0 $\downarrow$ )

2 weeks

901 (718 $\uparrow$  183 $\downarrow$ )



4 weeks

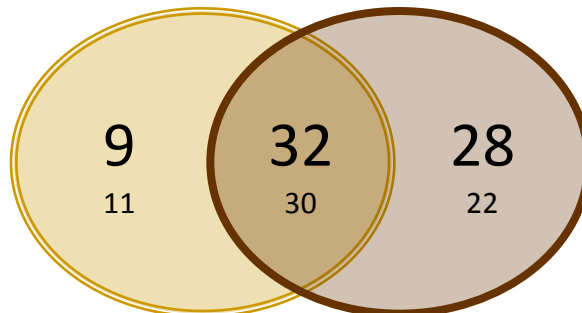
394 (390 $\uparrow$  4 $\downarrow$ )

41 ( $\uparrow$ )

60 ( $\uparrow$ )  
52 ( $\uparrow$ )

4 weeks

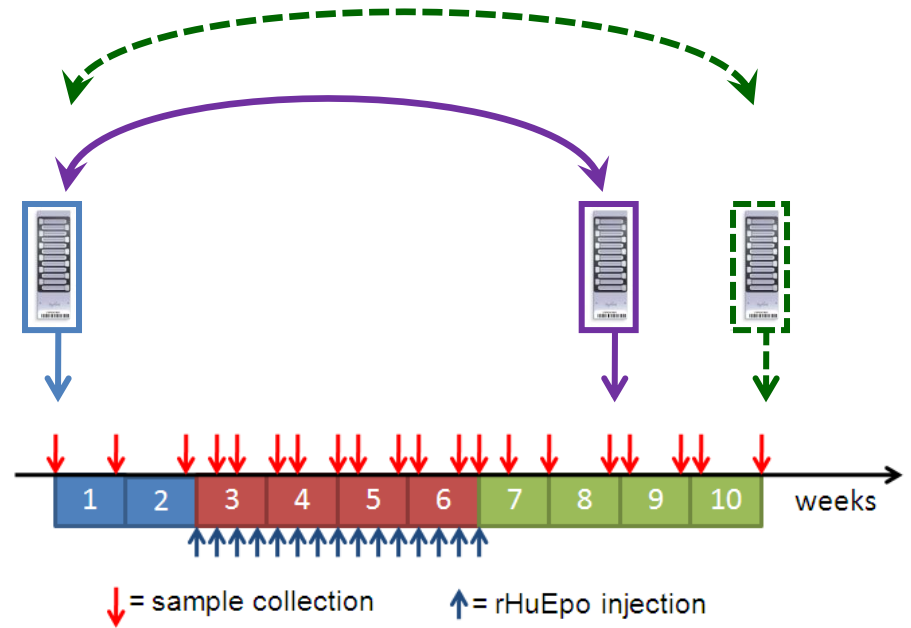
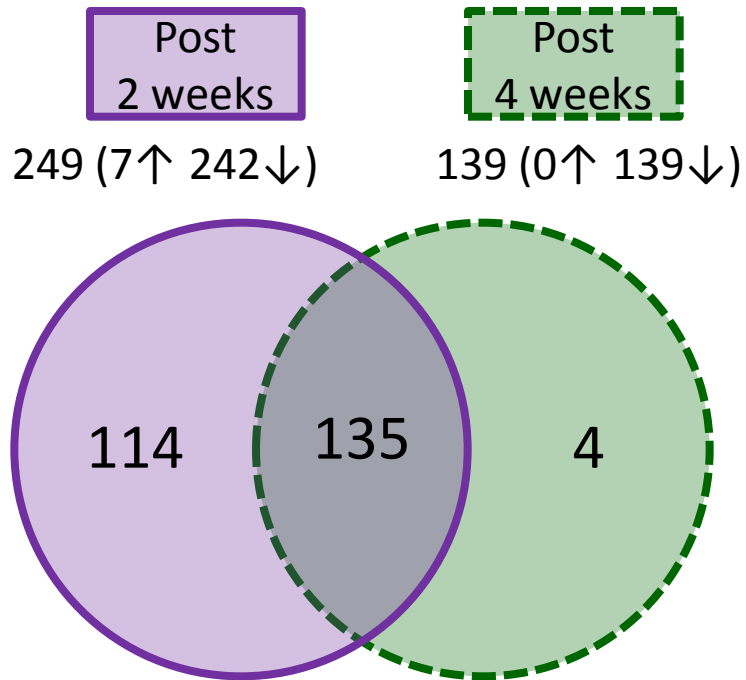
536 (533 $\uparrow$  3 $\downarrow$ )  
379 (377 $\uparrow$  2 $\downarrow$ )





5 % FDR significant and  $\geq 1.5$ FC: Post rHuEpo

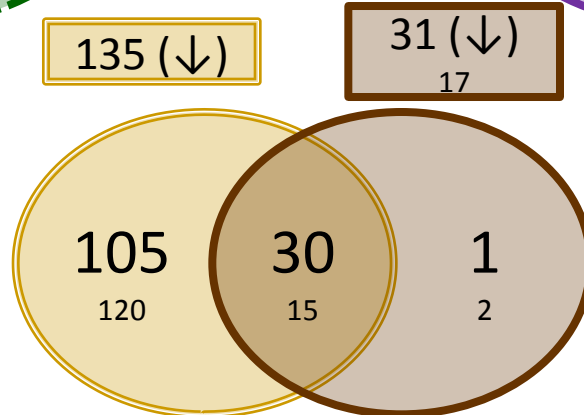
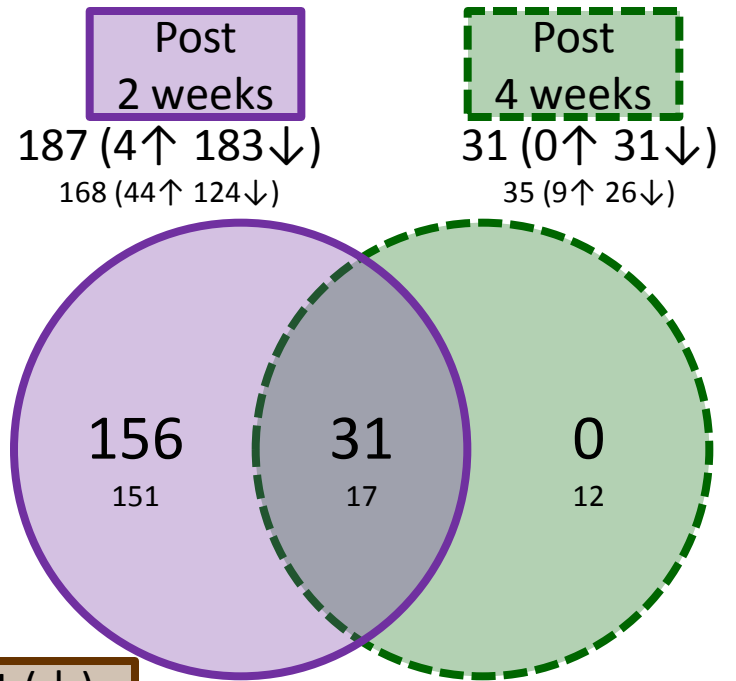
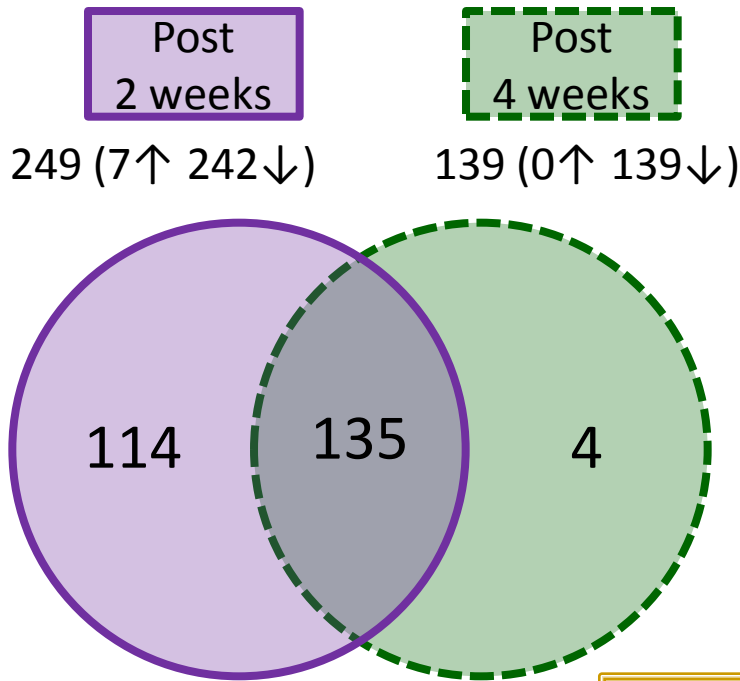
SCO (n=18)



5 % FDR significant and  $\geq 1.5FC$ : Post rHuEpo

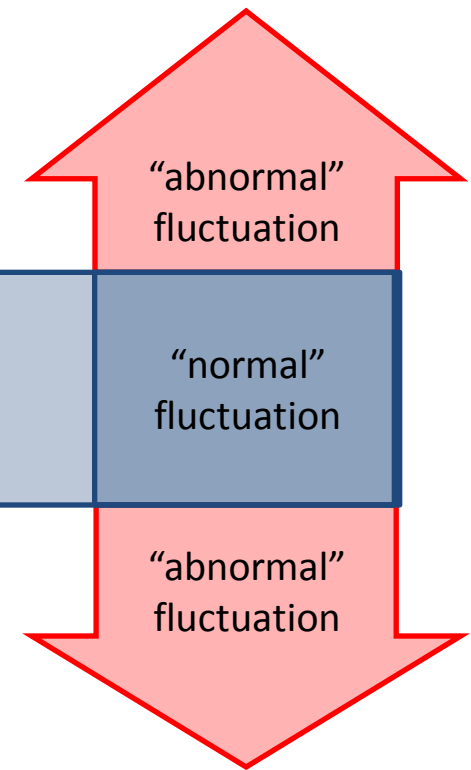
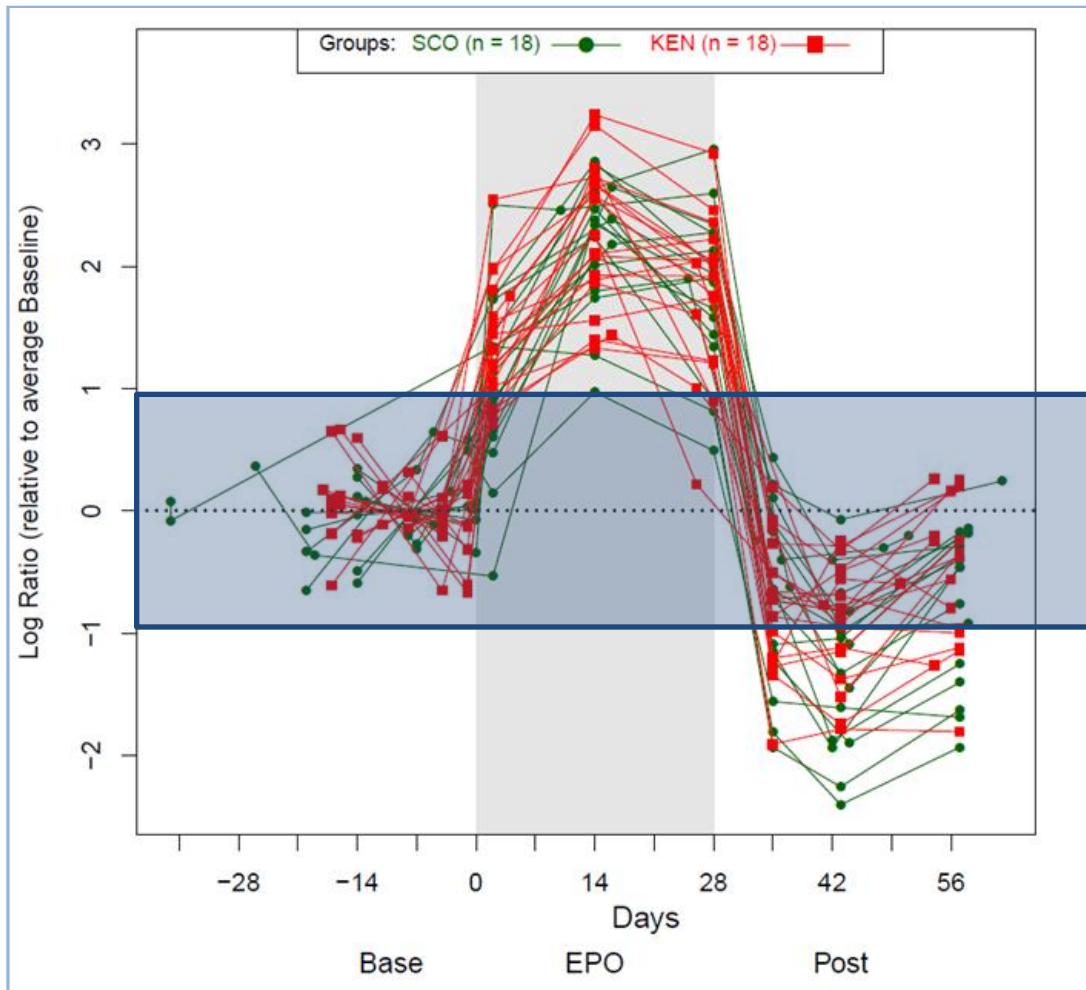
SCO (n=18)

KEN (n=18) (n=6)



# Future applicability?

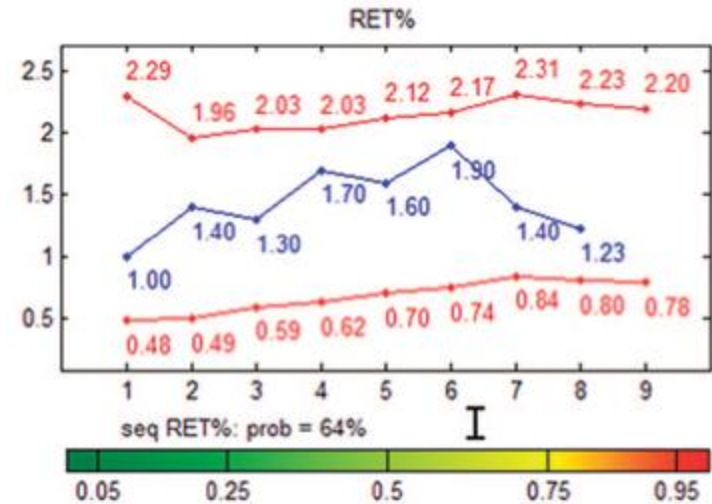
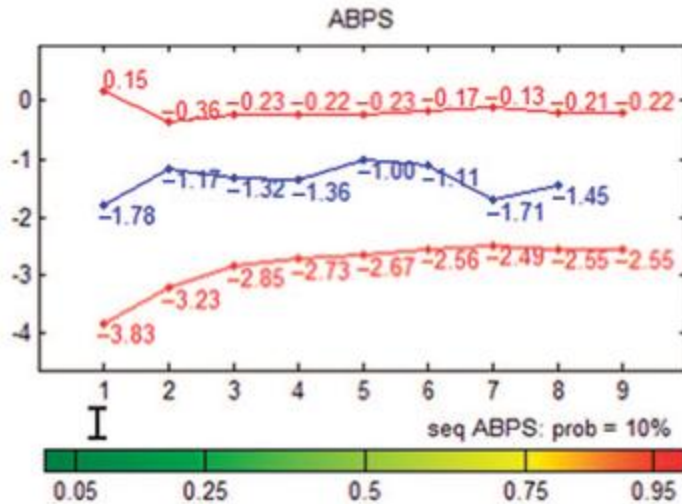
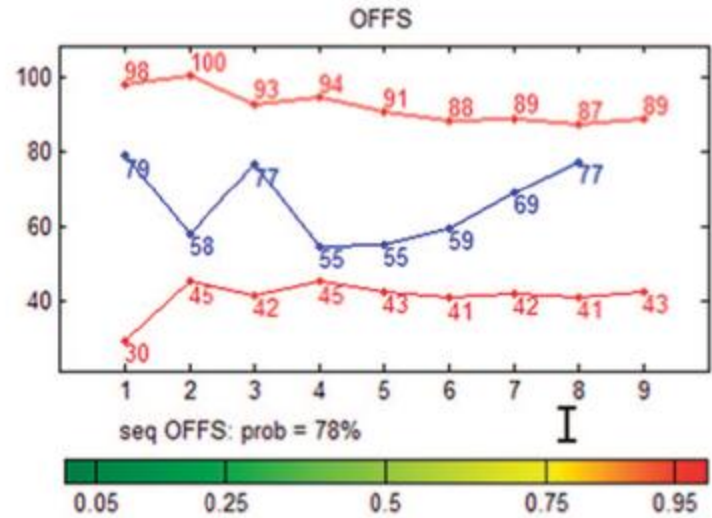
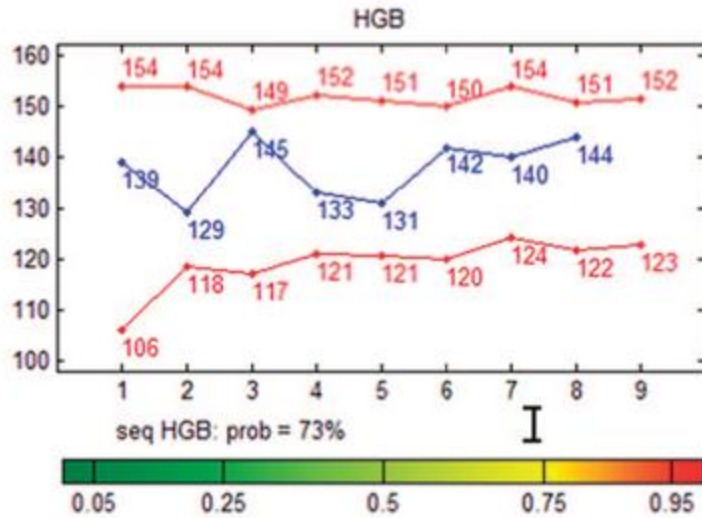
## The Athlete Biological Passport?



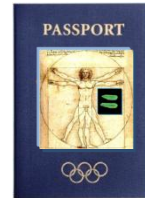
# Athlete **biological** passport?



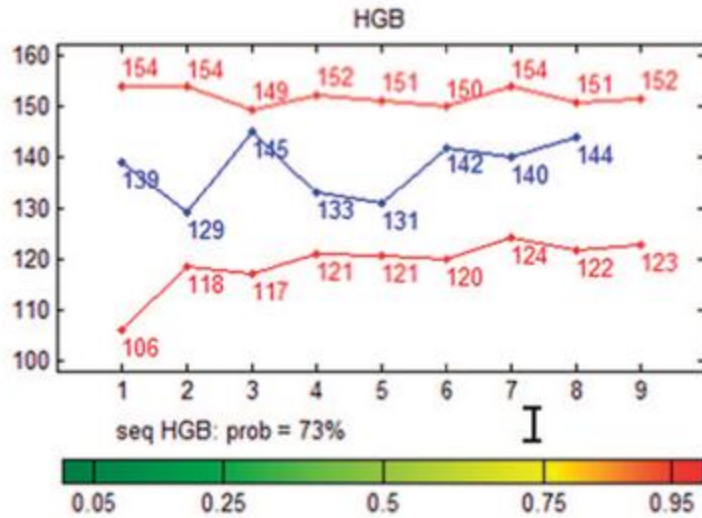
A



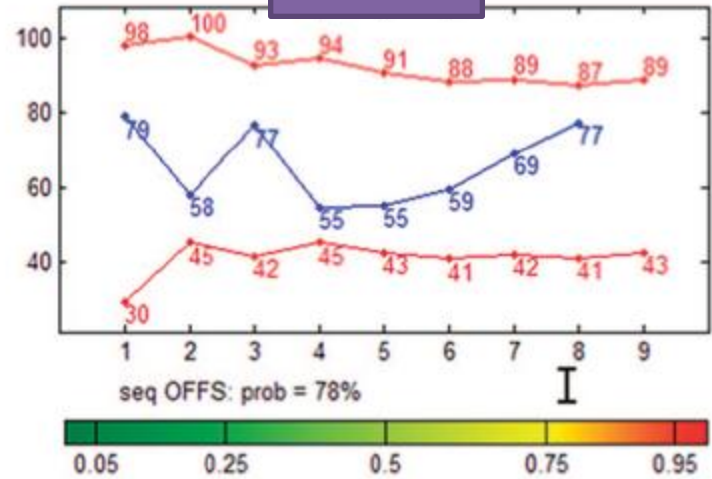
# Athlete **biological** passport?



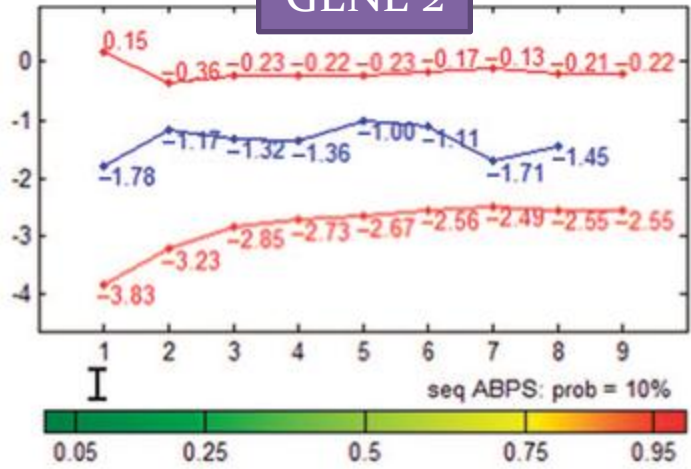
A



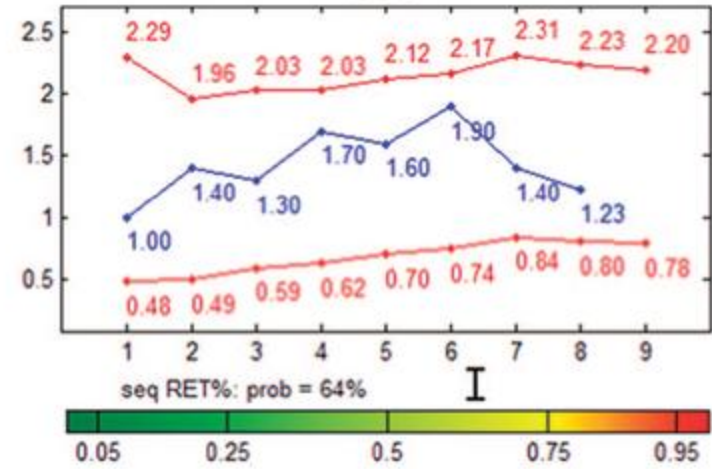
GENE 1



GENE 2



RET%



# Conclusions

- The first “molecular signature” of rHuEpo doping has been discovered.
- These results provide the strongest evidence to date that “OMICS” technologies such as gene expression have the potential to add a new dimension to currently applied ABP in terms of specificity and sensitivity for drug detection, not confined to rHuEpo.
- These very encouraging results serve to strongly reinforce the feasibility and need for this complex, expensive and technically demanding approach involving leading industry partners for the development of greatly improved detection methods.



“To those who devote their life to science, nothing can give more happiness than increasing the number of discoveries, but .. the cup of joy is full when the results of these studies immediately find practical applications”

**Dr Louis Pasteur (1822-1895)**

# Acknowledgments



**Mr. Jerome Durussel**  
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Ms. Wendy Crawford

Mr. Martin Anderson

Ms. Anne Keenan

Steering Committee members

The volunteers

