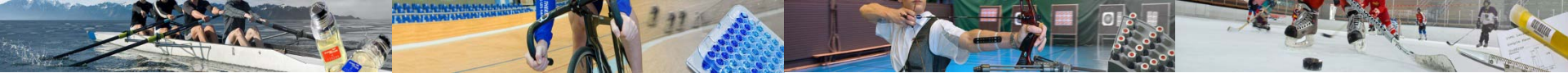


# The Athlete's Passport- Haematological Module

Martial Saugy

Swiss Laboratory for Doping Analyses  
Lausanne Switzerland

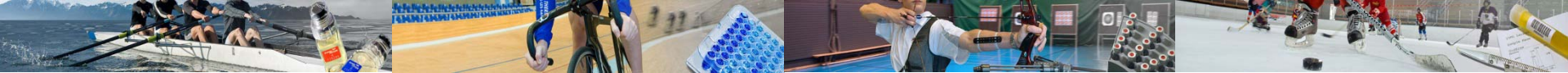




# The Athlete's Passport- Haematological Module

- Historical facts
- Principles of blood passport
- Process & Protocols
- Questions and Perspectives





## Historical facts

# 1993-94

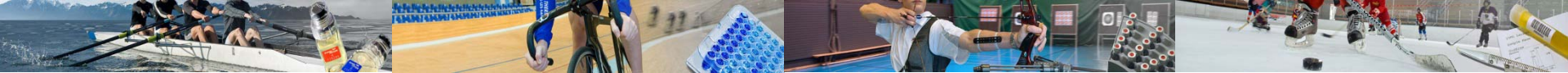
*Golden four  
IAAF meetings*

Birkeland, Donike, Ljungqvist,  
Fagerhol, Jenssen, Hemmersbach,  
Oftebro & Haug

*Int J Sports Med 1997: 18(1) 8.*

**Blood sampling in doping controls:  
First experiences from regular  
testing in athletics**





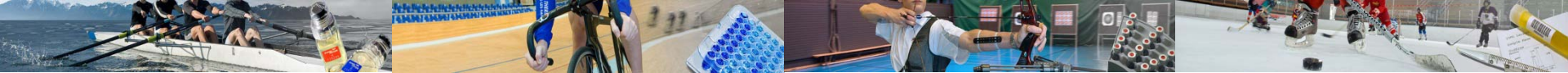
# Historical facts

## 1993-94



First blood sampling in Olympics:





## Historical facts

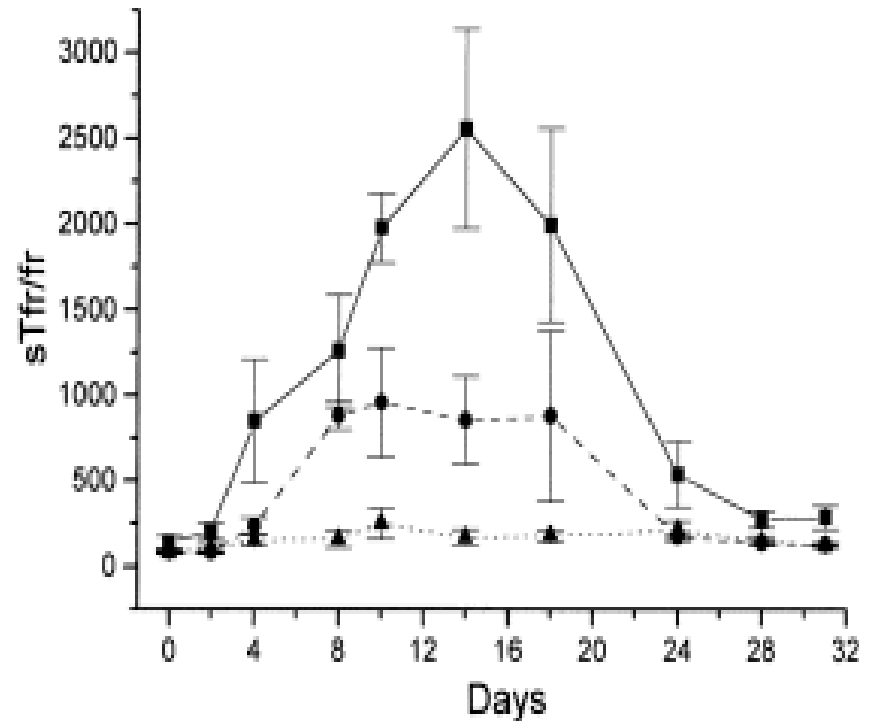
# 1996

Gareau, Audran, Baynes, Flowers,  
Duvallet, Sénécal & Brisson

*Nature, 1996, 380, 113*

Erythropoietin abuse in athletes

Ratio  
serum Transferrin receptor/  
Ferritin

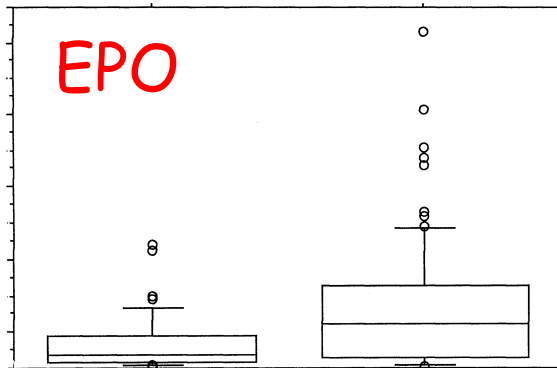




# Historical facts

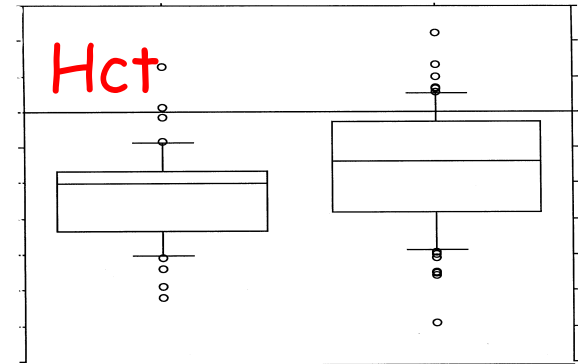
## 1996 Cycling

### Tour de Suisse experience



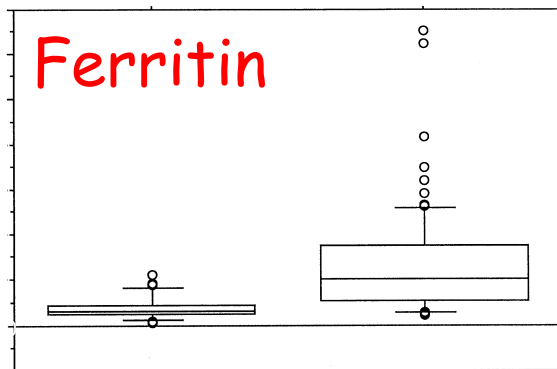
Controls

Cyclists



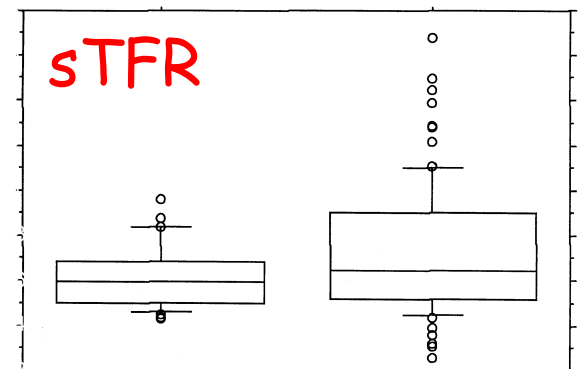
Controls

Cyclists



Controls

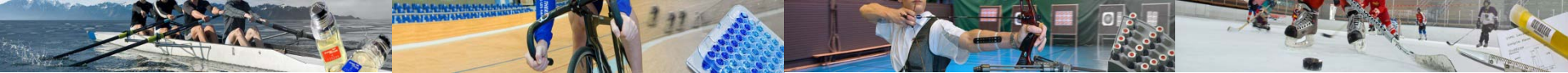
Cyclists



Controls

Cyclists





## Historical facts

# 1996-1997

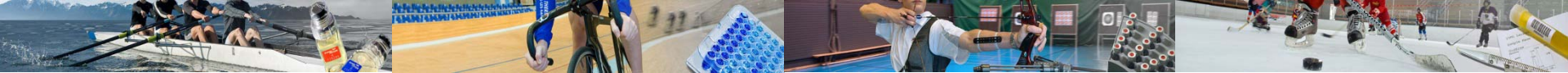
FIS and UCI  
Put threshold limits  
for resp. Hb and Hct:

**Paris-Nice March 1997:**

First "no Start":

Hematocrit: 57%





# Historical facts

## 2000

### The Australian Approach

Indirect with blood markers

On and Off score



Haematologica 2000; 85:564-572

original paper

Red Cells & Iron

### A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes

ROBIN PARISOTTO,\* CHRISTOPHER J. GORE,\* KERRY R. EMSLIE,° MICHAEL J. ASHENDEN,\* CARLO BRUGNARA,# CHRIS HOWE,° DAVID T MARTIN,\* GRAHAM J. TROUT,° ALLAN G. HAHN\*

\*Department of Physiology, Australian Institute of Sport, Canberra, Australia; °Australian Sports Drug Testing Laboratory, Australian Government Analytical Laboratories, Sydney, Australia; #Departments of Pathology and Laboratory Medicine, The Children's Hospital, Harvard Medical School, Boston, MA, USA

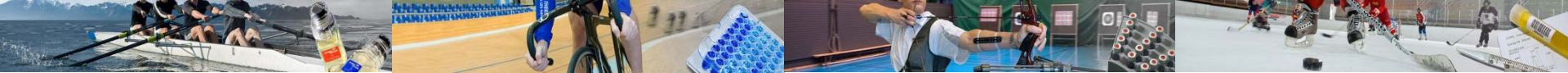
#### ABSTRACT

**Background and Objectives:** The use of recombinant human erythropoietin (r-HuEPO) to enhance athletic performance is prohibited. Existing tests cannot readily differentiate between exogenous and endogenous EPO. Therefore the aim of our study was to investigate possible indirect detection of r-HuEPO use via blood markers of altered erythropoiesis.

**Design and Methods.** Twenty-seven recreational athletes were assigned to three groups prior to a 25 day drug administration phase, with the following pro-

The use of recombinant human erythropoietin (r-HuEPO) is officially prohibited by the International Olympic Committee and other major sporting bodies.<sup>1</sup> However, currently available tests cannot readily differentiate between exogenous and endogenous EPO. Since r-HuEPO is demonstrably effective in increasing hemoglobin concentration [Hb], maximum oxygen consumption ( $\dot{V}O_{2max}$ ) and physical work capacity,<sup>2,3</sup> the lack of a test to confirm its use may have induced many athletes to experiment with the drug.





# Historical facts

## 2000

### The French Approach

### Direct: Urinary EPO

Lasne & de Ceaurriz

Recombinant erythropoietin in urine. *Nature* 2000 405(6787):635

**brief communications**

## Recombinant erythropoietin in urine

An artificial hormone taken to boost athletic performance can now be detected.

**E**rythropoietin is a hormone that stimulates the production of new red blood cells (erythropoiesis). Although athletes use recombinant human erythropoietin illicitly to boost the delivery of oxygen to the tissues and enhance their performance in endurance sports, this widespread doping practice cannot be controlled in the absence of a reliable analytical procedure to monitor it. Here we describe a new technique for detecting this drug in urine following its recent administration.

The stimulation of erythropoiesis by erythropoietin (EPO) makes this drug very attractive to sportspeople wishing to improve their aerobic power, although the International Olympic Committee banned its misuse ten years ago. Detection has been a problem — analysis of haematological<sup>1</sup> or biochemical<sup>2</sup> parameters indicates only that erythropoiesis has been stimulated, but cannot confirm that drug administration is to blame.

To detect administered hormone directly

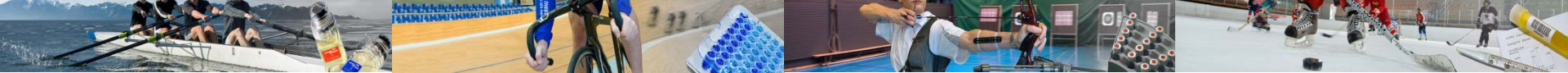
**Figure 1** Autoradiograph of isoelectric patterns of exogenous and endogenous erythropoietin (EPO). Images were obtained by chemiluminescent immunodetection of blotted EPO after isoelectric focusing. **a**, Purified commercial human urinary natural EPO (Sigma); **b**, recombinant EPO- $\beta$  (Neorecomon, France); **c**, recombinant EPO- $\alpha$  (Eprex, France); **d**, urine from a control subject; **e, f**, urine from two patients treated with Neorecomon EPO for post-haemorrhagic anaemia; **g, h**, urine from two cyclists from Tour de France 1998 (samples concentrated by ultrafiltration). Note the 'mixed' appearance of the pattern in **e**. The cathode is at the top; pH values are indicated on the left.

the presence of recombinant isoforms, and sometimes acidic bands as well, depending on the presence of endogenous isoforms. The presence of exogenous hormone was always evident: any individual injected with recombinant EPO showed a striking transformation of their initial EPO urine pattern. We assayed 102 frozen urine samples

selected these samples for isoelectric focusing as they were more likely to contain exogenous hormone; indeed, they all gave rise to a banding pattern typical of recombinant hormone.

Our method for detecting recent exposure to recombinant EPO in athletes could be useful for in-competition controls in

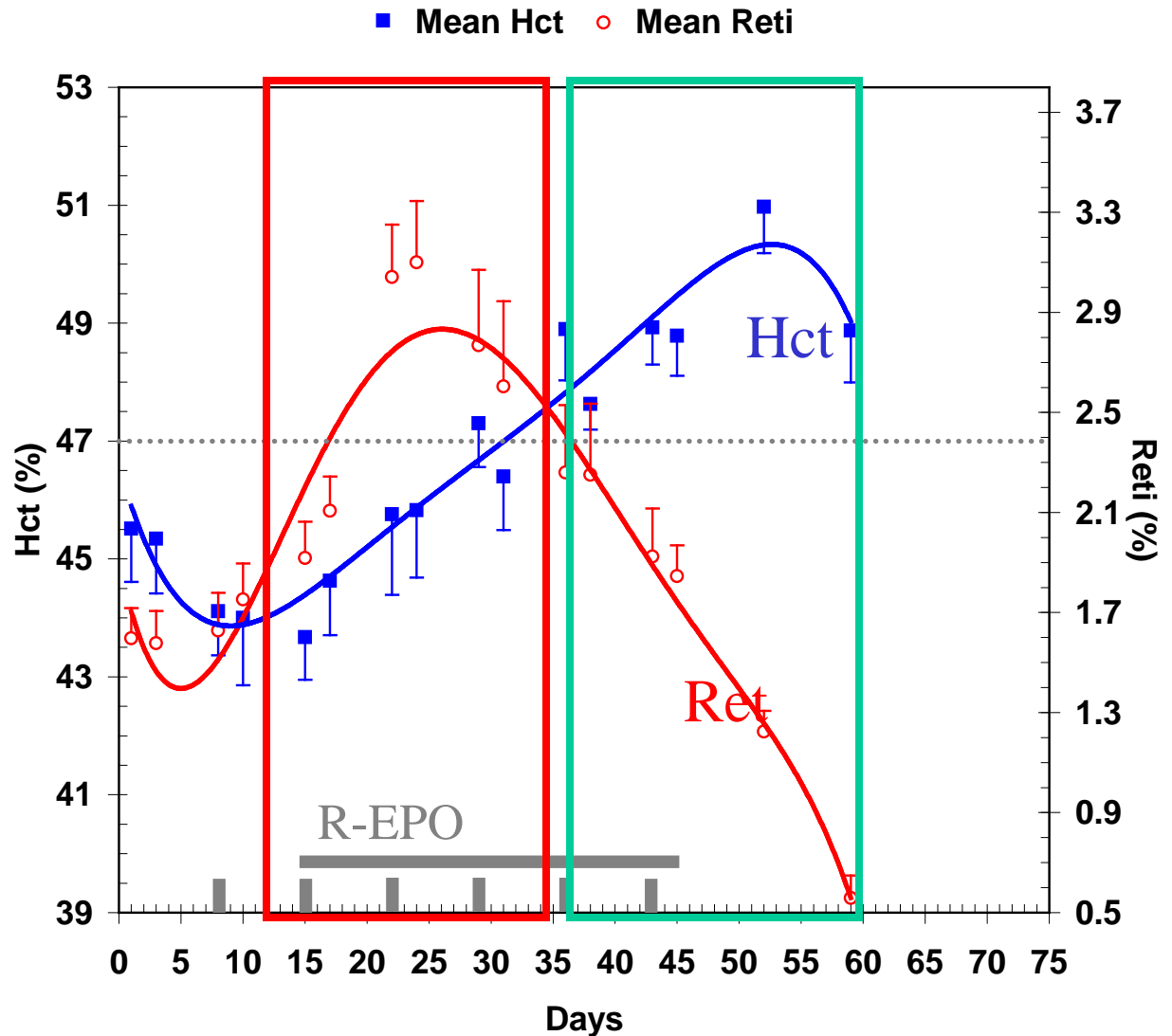


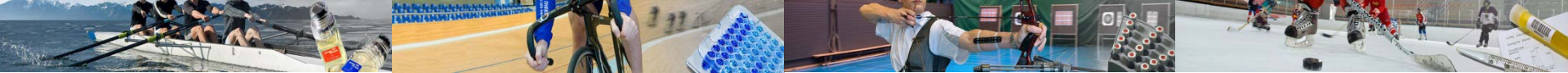


# Historical facts

2001 &..

On  
And  
Off





# Historical facts

## 2003

Sport Hematology

Decision Making and Problem Solving



### Hematologic passport for athletes competing in endurance sports: a feasibility study

LUCA MALCOVATI, CRISTIANA PASCUTTO, MARIO CAZZOLA

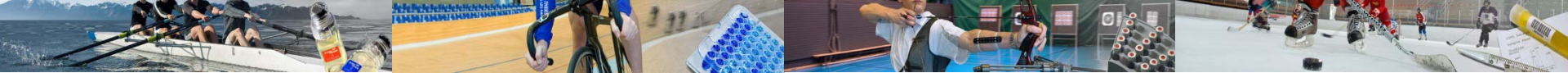
Malcovati  
Pascutto  
Cazzola

*Background and Objectives.* Strategies based on the use of upper thresholds of hemoglobin or hematocrit to detect blood doping in endurance sports have essentially failed to deter this malpractice. With the aim of establishing a more effective strategy, we analyzed the biological variations of hematologic parameters in professional athletes and investigated the possibility of defining subject-specific reference ranges that could distinguish between physiologic and abnormal variability.

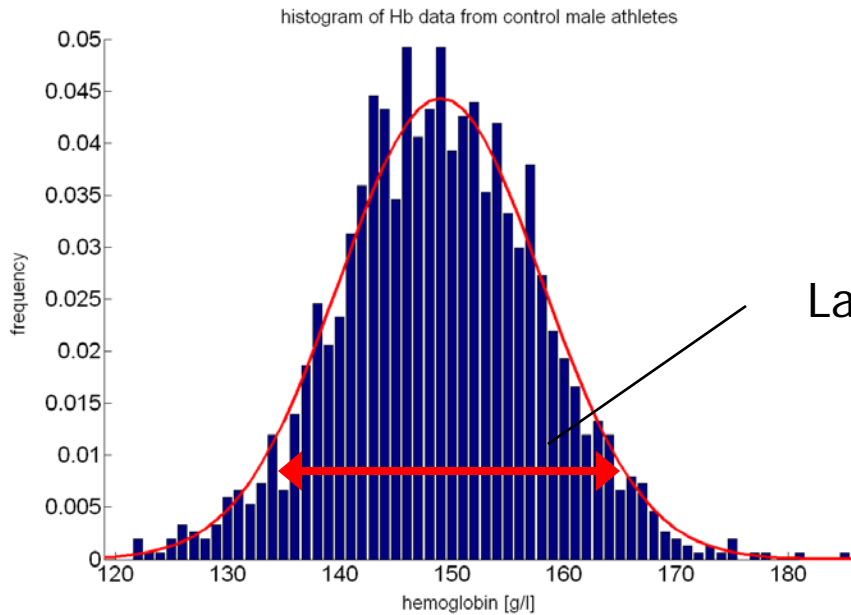
*Design and Methods.* Hemoglobin concentration, hematocrit, reticulocyte count, serum ferritin and soluble transferrin receptor levels were sequentially evaluated in 923 professional football players. Using the analysis of variance we tested the effect of age, ethnicity, exercise modalities and training phases on hematologic parameters and then estimated components of variation. The significance of the difference between two measures was obtained from the distribution of the within-subject variance (the

**B**lood doping is an illicit practice aimed at increasing tissue oxygen delivery and aerobic performance, mainly but not exclusively in endurance sports. Over the decades, various approaches have been used, including homologous<sup>1</sup> and autologous<sup>2</sup> red blood cell transfusion, recombinant human erythropoietin (rHuEpo) or related erythropoietic stimulants,<sup>3</sup> rHuEpo-enhanced autologous transfusion,<sup>4</sup> and hemoglobin-based oxygen carriers (HBOCs).<sup>3</sup> Although blood doping is particularly exploited in endurance disciplines (e.g. cycling, track and field events, and cross-country skiing), it can confer significant advantage in recovery after intermittent efforts<sup>3</sup> and, therefore, other disciplines must also be considered at risk of this practice.<sup>5</sup>





# Principles of blood passport



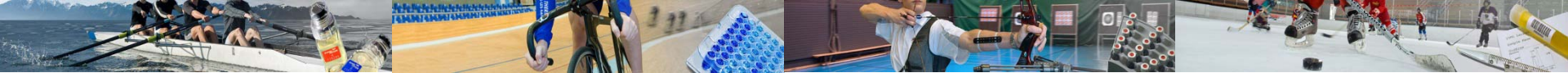
Large between-rider variance

Hgb

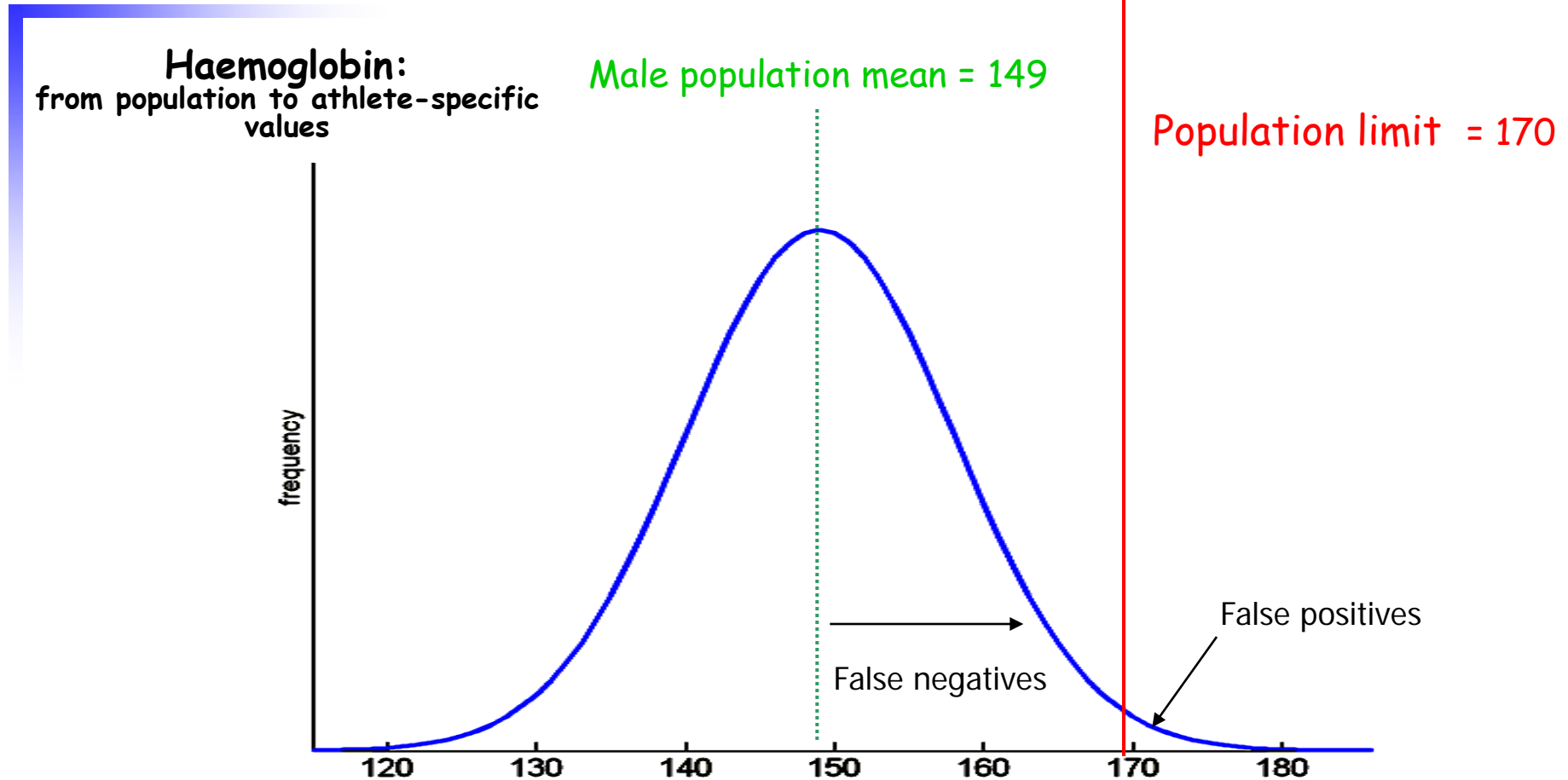
Population mean value is 149 g/L for males and 133 g/L for females

Between-rider variance = 57.2g/L

Within-rider variance = 28.2g/L



# Principles of blood passport

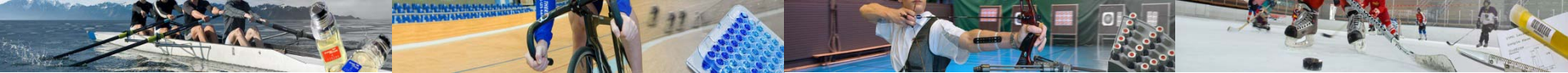


**Current Threshold at 170 g/l:**

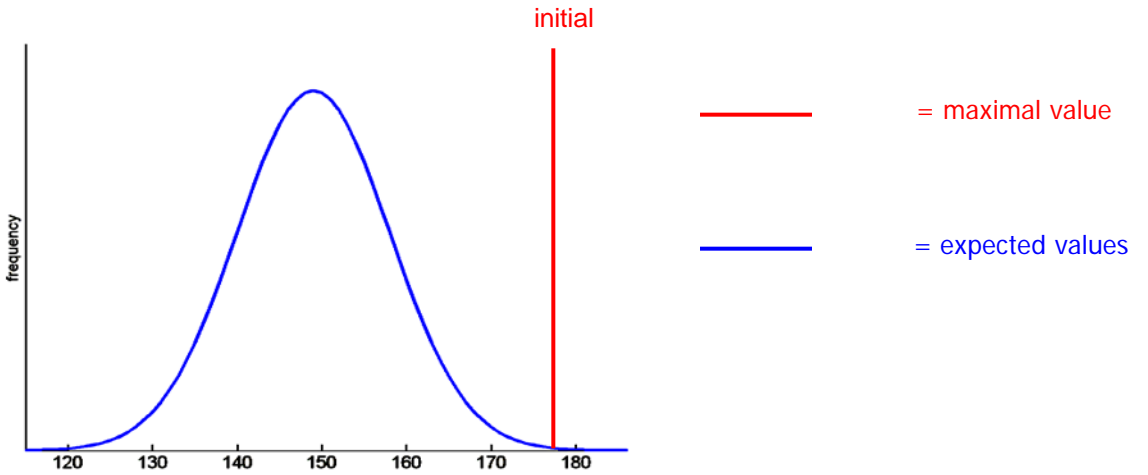
Much room for many athletes to dope and monitor their Hgb profile -  
*very low sensitivity*

Still some false positives - *low specificity*

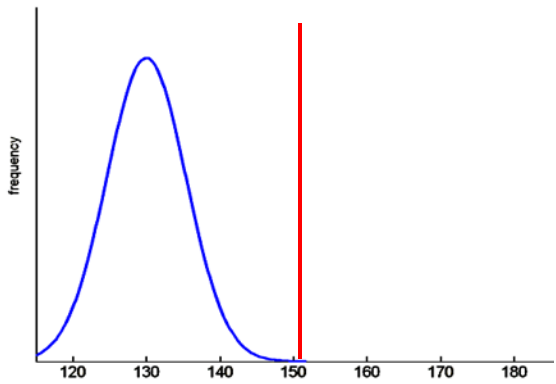
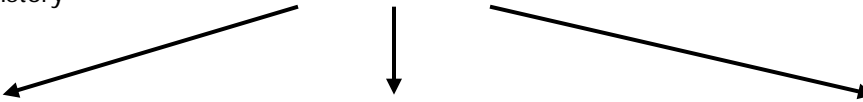




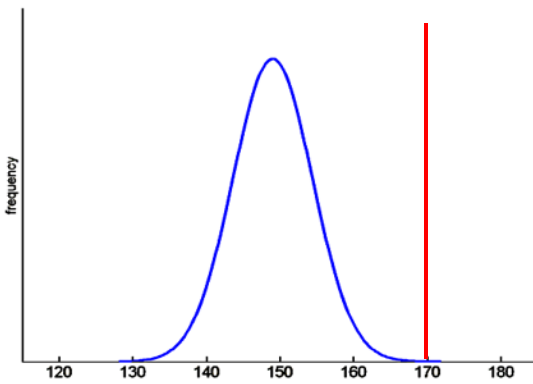
# Haemoglobin: from population to athlete- specific values



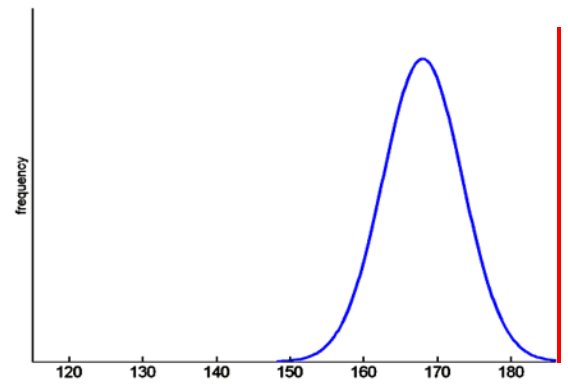
differentiation based on previous test history



athlete with naturally low haemoglobin

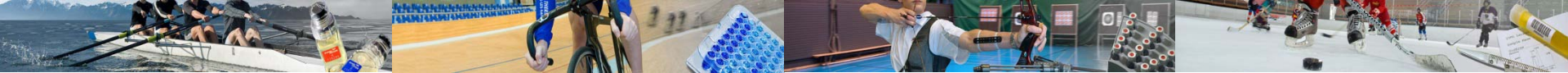


athlete with medium haemoglobin



athlete with naturally high haemoglobin





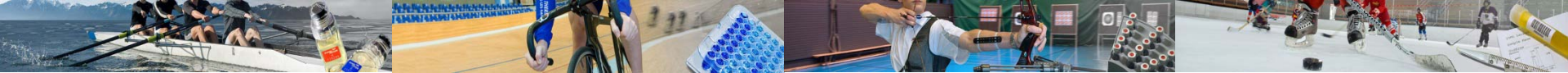
# Principles of blood passport

## Serial monitoring and athlete-specific values

- AAF type I: Abnormal new observation
- AAF type II: Abnormal sequence of observation

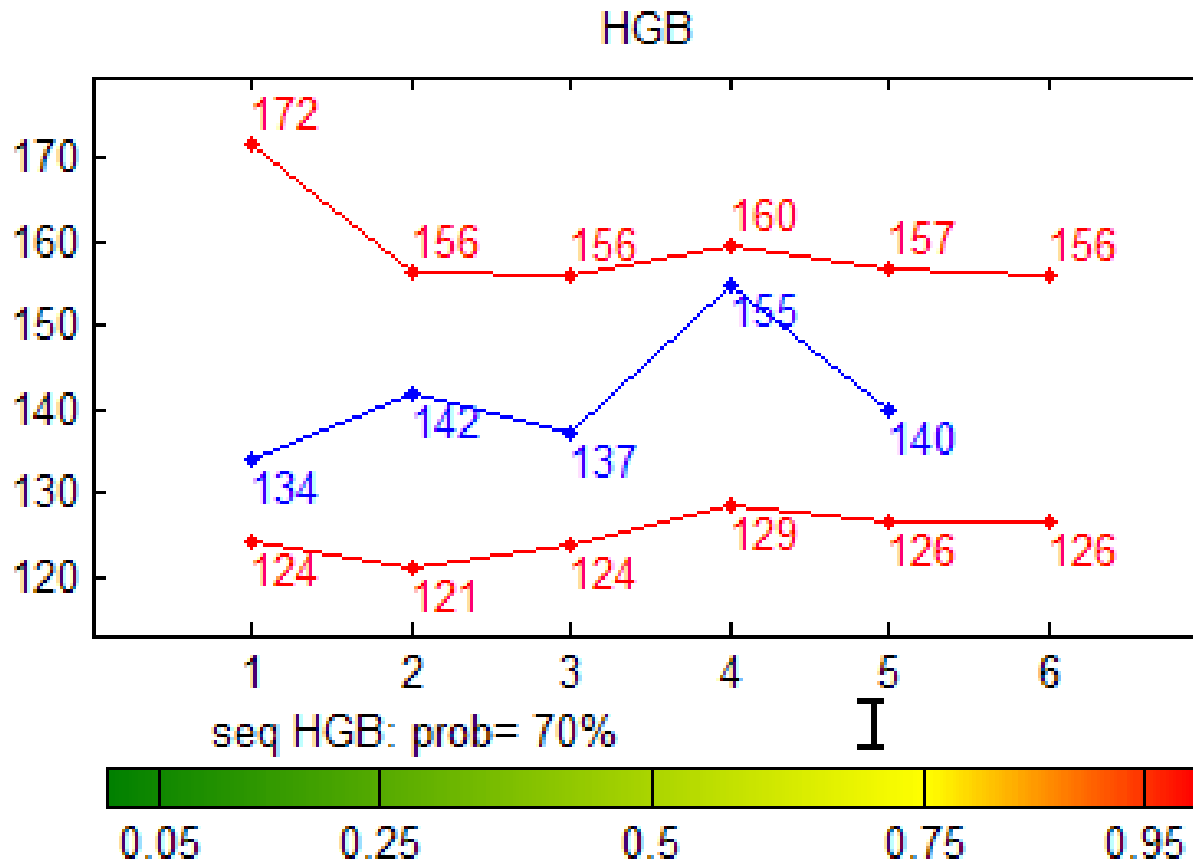
## Heterogeneity tables

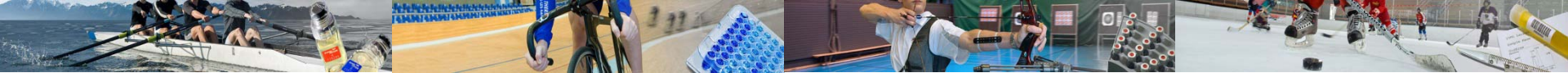
- There are six heterogeneous/confounding factors for HGB and OFFS:
- (1) gender (fixed factor)
- (2) ethnic origin (fixed factor)
- (3) age (fixed factor)
- (4) altitude (time-varying factor)
- (5) type of sport (fixed factor)
- (6) Instrument related technology (time-varying factor)



# Principles of blood passport

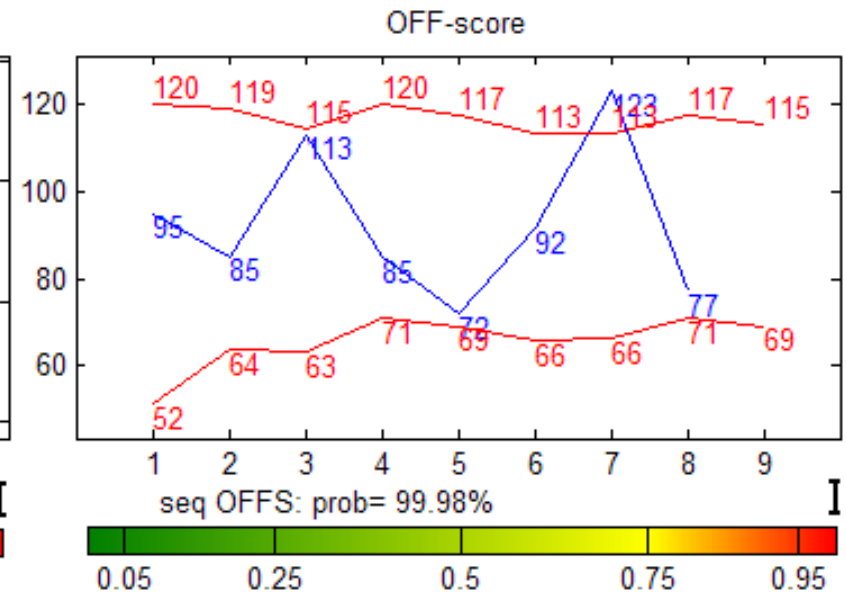
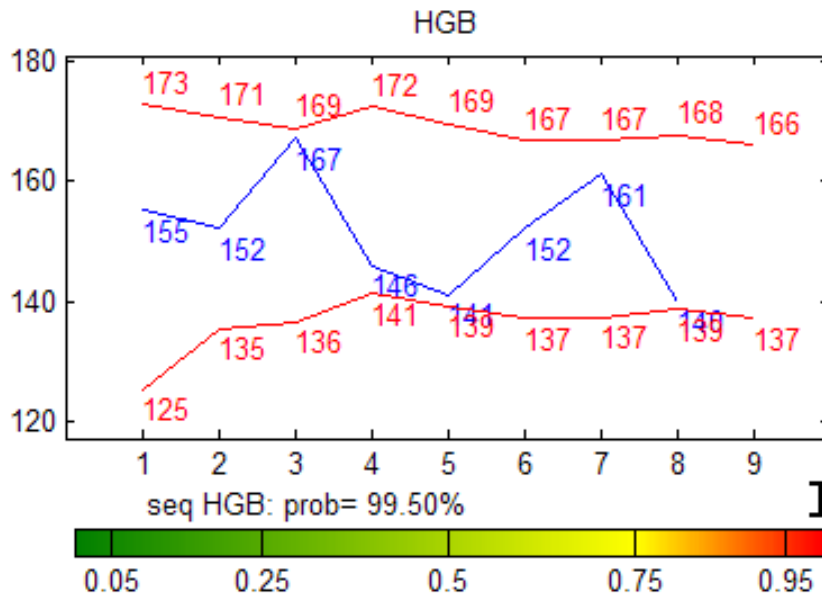
## Example of normal sequence

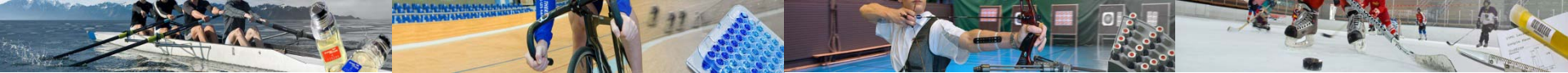




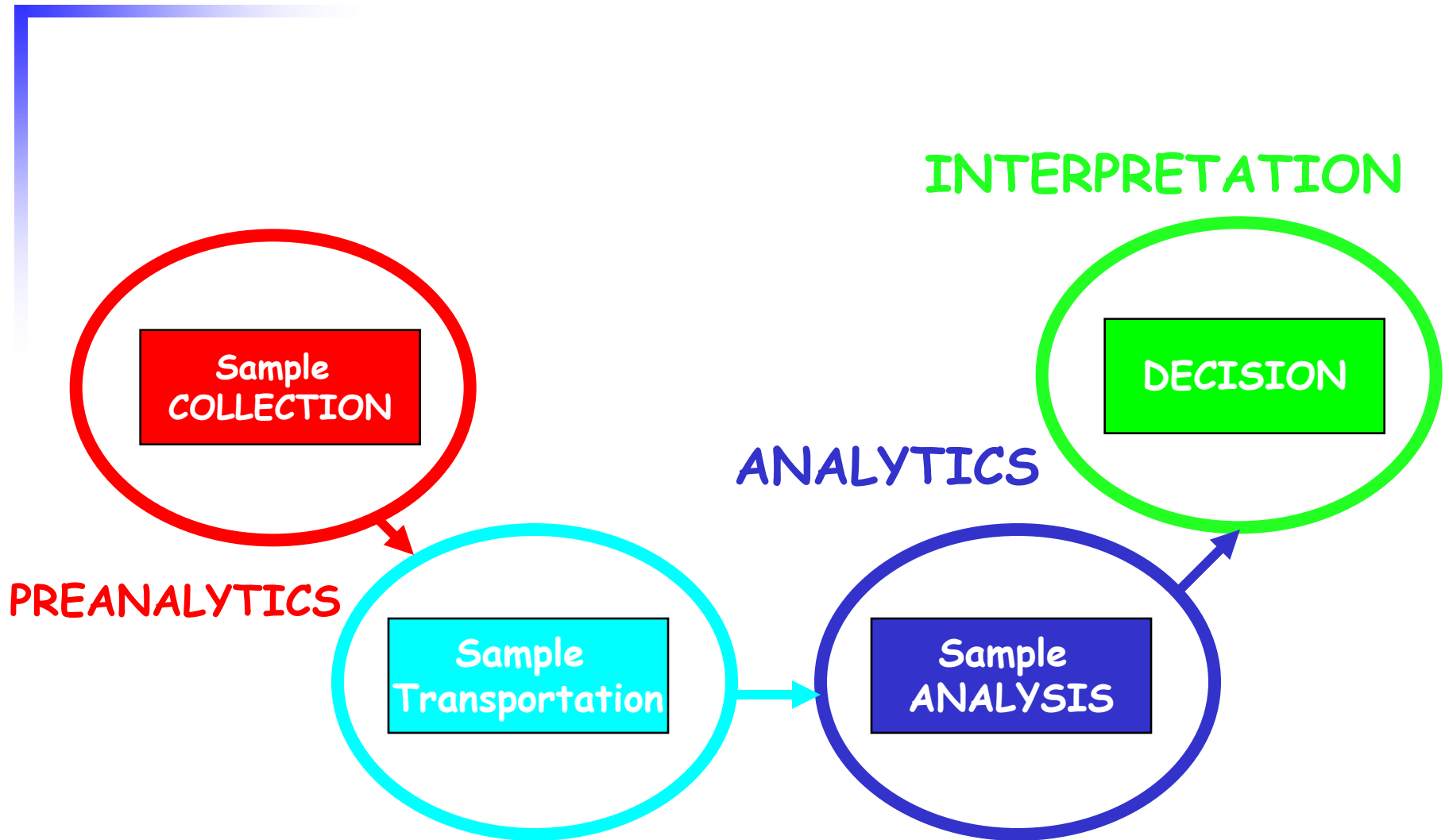
# Principles of blood passport

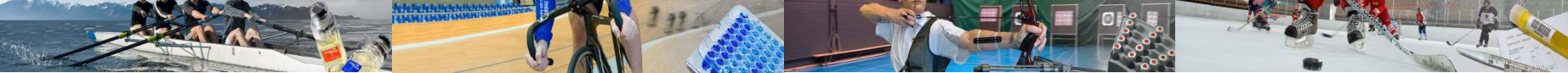
## Example of abnormal sequences





# Process and Protocols



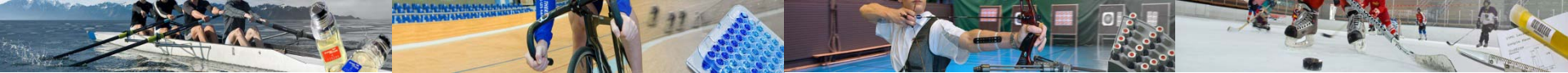


# Process and Protocols

## Collection

1. Introduction (IST)
2. Doping control station
3. Timing of sample collection > 2h after training
4. 10 min time out
5. Doping control form (altitude, hypoxic dev., training,...)
6. Sample collection equipment
7. Sample collection procedure
8. Homogenization
9. Application of dressing
10. Identification of the tube
11. Signing blood collection form
12. Sealing blood sample
13. Troubleshooting





# Process and Protocols

## Storage & Transport

### 1. Introduction (IST)

### 2. Storage procedure

- Type of storage devices
- Capabilities of storage devices (2-12 deg, data logger)
- Security of storage device

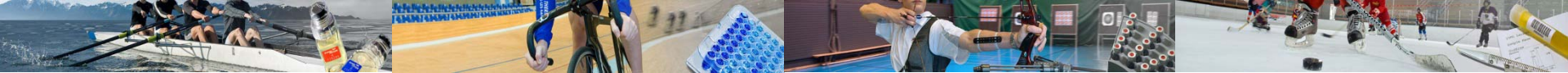
### 3. Transport procedure

- Type of transport devices
- Capabilities of transport devices (2-12 deg, data logger)
- Security of transport devices

#### *Remarks concerning transport procedure*

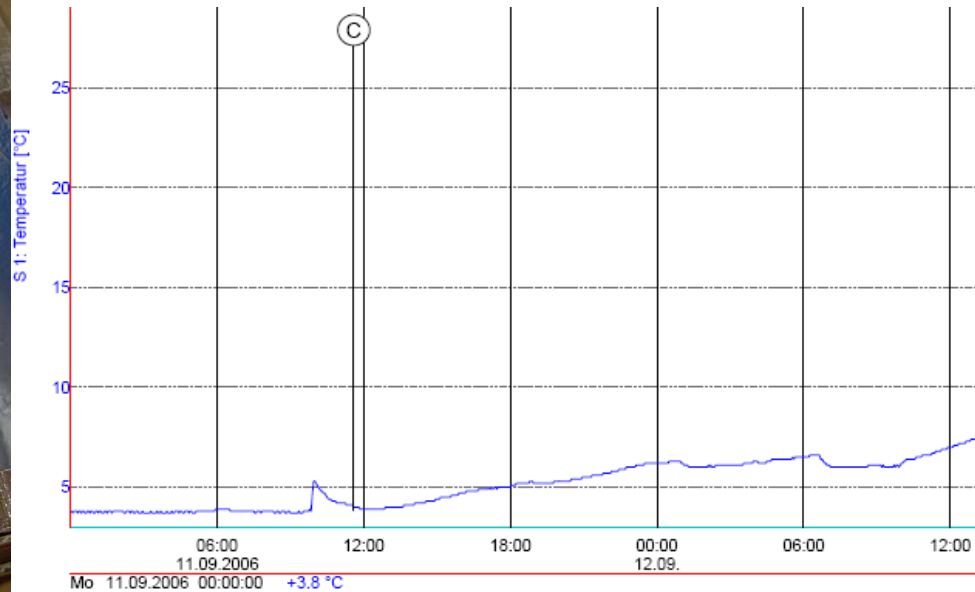
- *If < 2h, 5 to 25 deg allowed*
- *Analysis in less than 36 hours*

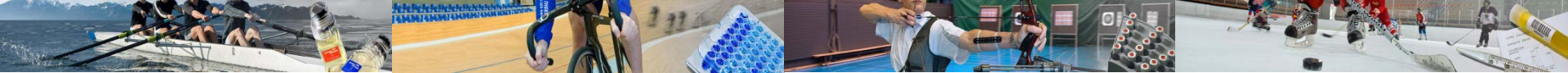




# Process and Protocols

## Storage & Transport





# Process and Protocols

## Analytical

### 1. Introduction

- IC & OOC
- ISL applicable, if discrepancy, BAP shall prevail
- All terms defined in "Code", ISL & IST.
- Satellite def: Mobile unit or Hem.Lab. WADA approved of a WADA accr. Lab.

### 2. Analytical procedure

- Network of WADA accredited labs or satellite labs
- Instrument checks
  - internal controls
  - Follow manufacturer recommendations





**Negative**

Sample No.  Birth  Ward  Date   
 Pat. ID  Sex  Dr.  Time   
 Name  Comment

Main **Graph** WBC RBC Cumulative Q-Flags Service Research(W) Research(R)

Items

Item	Data	Unit
WBC	5.85 *	10 <sup>3</sup> /uL
RBC	5.07	10 <sup>6</sup> /uL
HGB	16.0	g/dL
HCT	47.0	%
MCV	92.7	fL
MCH	31.6	pg
MCHC	34.0	g/dL
PLT	163	10 <sup>3</sup> /uL
RDW-SD	49.7	fL
RDW-CV	14.9	%
PDW	11.2	fL
MPV	10.1	fL
P-LCR	25.5	%
PCT	0.17	%
RET%	0.09	%
RET#	0.0046	10 <sup>6</sup> /uL
IRF	0.0	%
LFR	100.0	%
MFR	0.0	%
HFR	0.0	%

WBC Differential

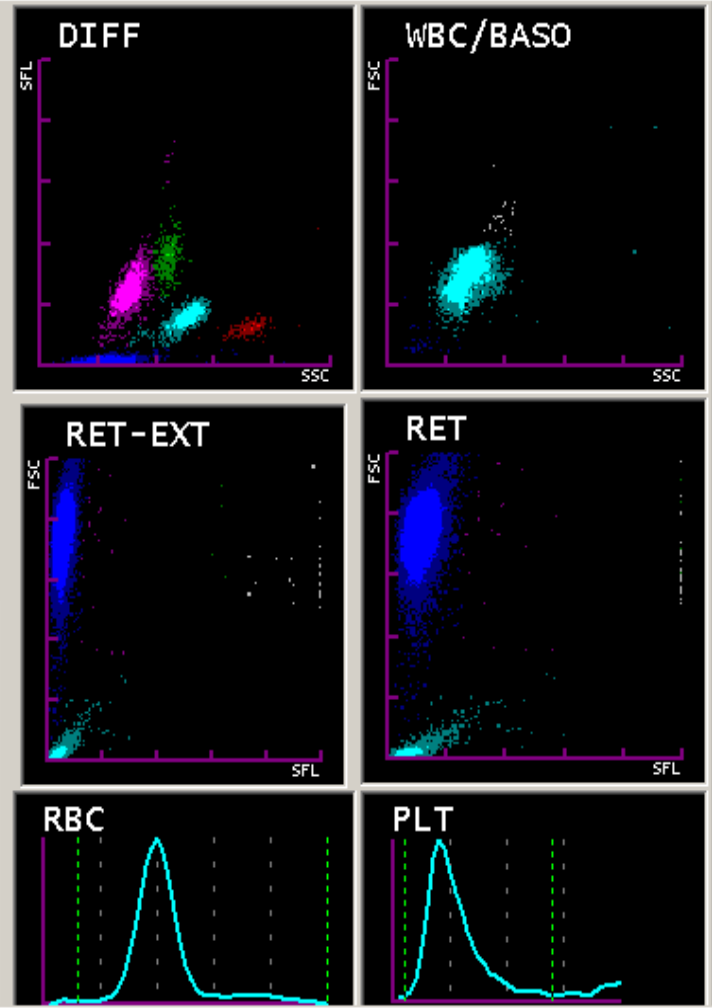
Item	Data	Unit
NEUT#	1.75 *	10 <sup>3</sup> /uL
LYMPH#	3.40 *	10 <sup>3</sup> /uL
MONO#	0.42 *	10 <sup>3</sup> /uL
EO#	0.27 *	10 <sup>3</sup> /uL
BASO#	0.01 *	10 <sup>3</sup> /uL
NEUT%	29.9 *	%
LYMPH%	58.1 *	%
MONO%	7.2 *	%
EO%	4.6 *	%
BASO%	0.2 *	%

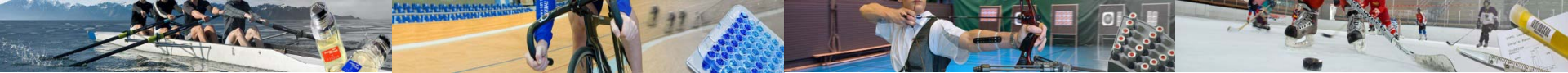
Flag(s)

WBC: NRBC?

RBC/RET:

PLT:





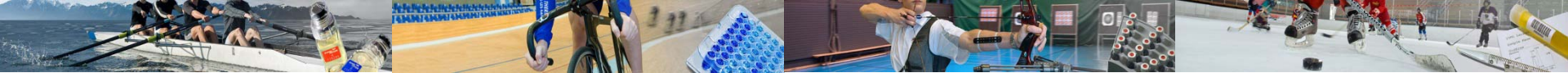
# Process and Protocols

## Interpretation

### 1. Introduction

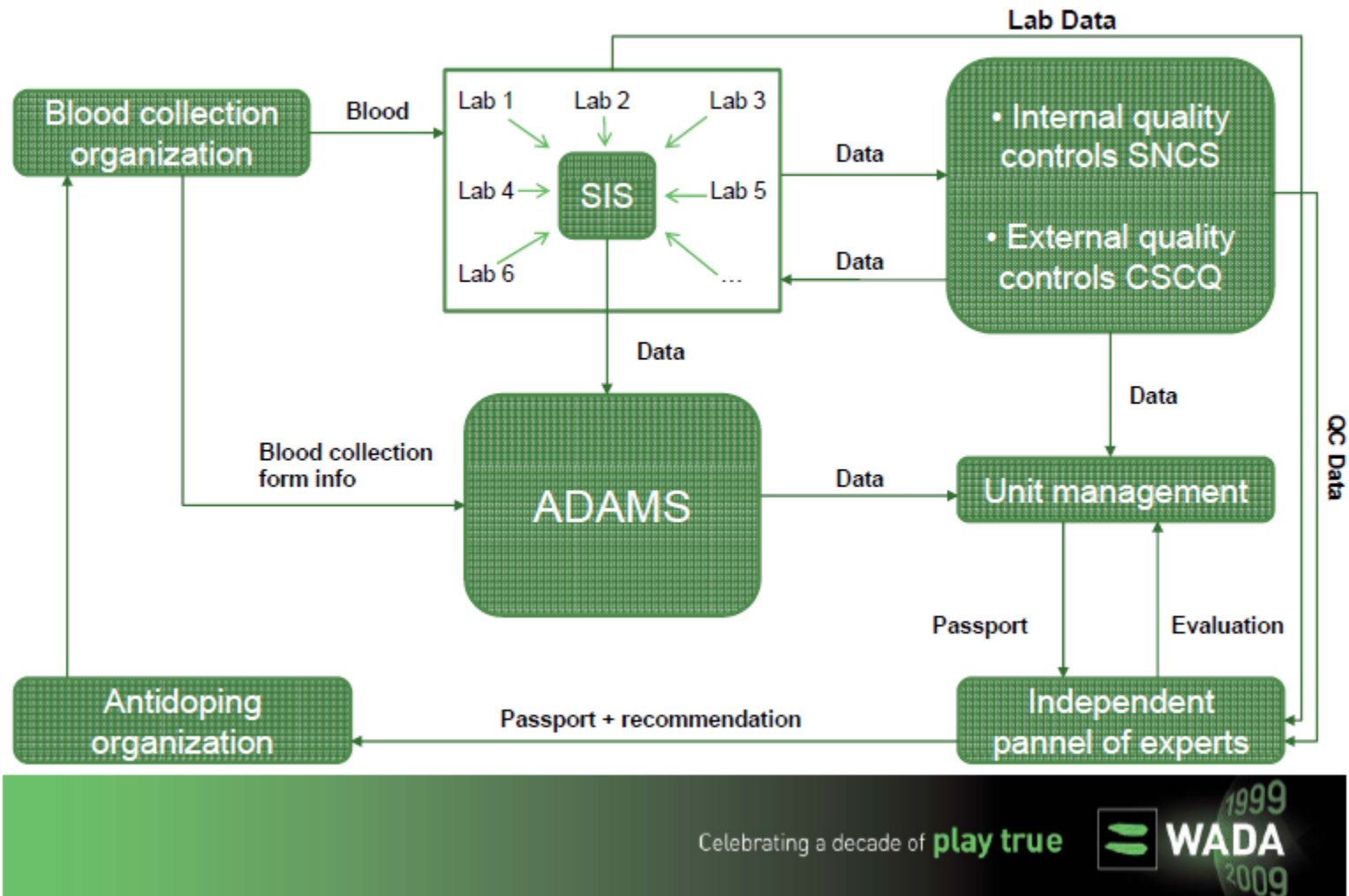
### 2. Definitions

- Longitudinal data (RES\_N)
- Population mean (POP\_ME)
- Between & Within Subject Variance (BS\_ or WS\_VAR)
- Specificity (% of negatives correctly identified)
- Expected & presenting values
- Minimal & maximal values
- Likelihood function of a sequence
- Expected sequence and maximal threshold
- Heterogeneous and confounding factors (time varying or fixed)



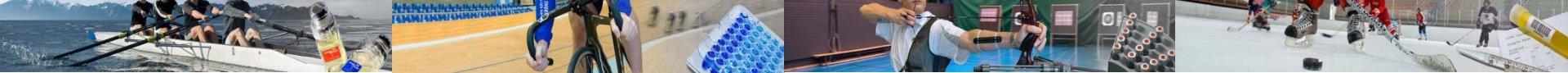
# Athlete Passport Process

Wada accredited laboratories



Celebrating a decade of **play true**

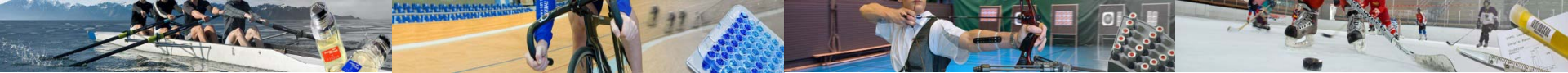




# Questions & Perspectives

	<b>direct</b> test for the <u>presence</u> of an exogenous substance	<b>indirect</b> test for <u>disturbances</u> in the body <u>caused</u> by the intake of a substance
<i>main advantages:</i>	<ul style="list-style-type: none"><li>• <i>easier to interpret: yes or no decision</i></li><li>• <i>actual substance is identified</i></li></ul>	<ul style="list-style-type: none"><li>• <i>can test any substance or method</i></li><li>• <i>the same methodology can be applied for the detection of all substances</i></li></ul>
<i>main drawbacks:</i>	<ul style="list-style-type: none"><li>• <i>cannot test for substances with same molecular structure as endogenous</i></li><li>• <i>a new test must be developed for any new substance</i></li></ul>	<ul style="list-style-type: none"><li>• <i>harder to interpret: has to deal with natural variability</i></li><li>• <i>doping substance or method may be unknown</i></li></ul>





## Questions & Perspectives

### Which rules ?

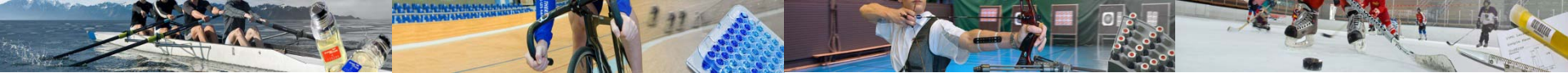
#### 1. Competition rule:

“No-start” or ineligibility , temporary suspension with a specificity of 99% ?

#### 2. Anti-doping infraction:

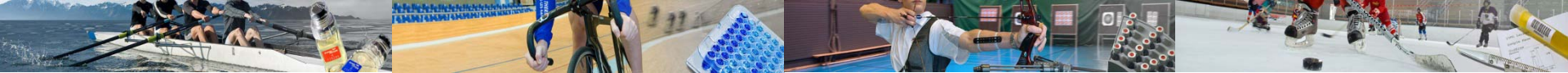
“AAF”, Application of WADA code with a specificity of 99.9%





## Questions & Perspectives

- Applicable to all sports federations/countries?
- Blood collection
  - Education of BCOs !
  - Audit the Blood Collection Organisations
  - Applicability of IST ?
- Blood transport
  - Price !!!
  - import/export biological samples.
  - better use of several already existing networks



## Questions & Perspectives

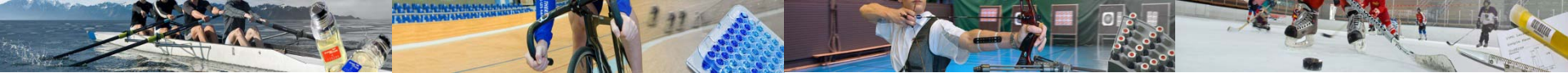
### - Analysis

- good laboratory network !
- good quality system
- mobile units & satellite labs?
- 7 days availability (clinical lab)
- immediate report
- hardly any return of investment
- Documentation packages

### - Interpretation

- role of the expert commission
- education of disciplinary panels
- target test, than additional information to CAS?





Thanks to

**Neil Robinson & Pierre-Edouard Sottas**

for the preparation of this presentation

and Thank'you !

