

- **Note that the opinions expressed within the abstracts and presentations are those of the authors/presenters alone and do not necessarily reflect the opinions of WADA.**

Surrogate markers for Transgene Expression – Global Gene Expression

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About HFL

- Sports testing (since 1963)
 - **Pharmaceutical / Healthcare**
- 130 people
- State-of-the-art facilities (1997)
- Analytical laboratory
 - **method development/validation**
 - **complex matrices**
 - **biomarkers & the “omics”**



Drug testing in sport

Direct test of drug in blood / urine sample

TEST FOR:

- Drugs
- Metabolites



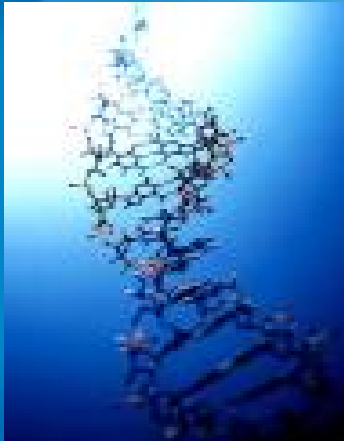
CONVENTIONAL ANALYTICAL TECHNIQUES:

- TLC/HPLC
- GC-MSⁿ
- LC-MSⁿ
- ELISA / RIA

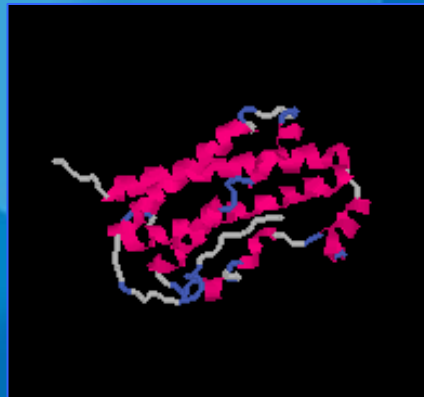
Difficulties with this testing approach?



**NEW
PHARMACEUTICAL
DRUGS**



Gene therapy



Proteins/peptides



Designer drugs

Gene Therapy to Gene Doping

Non-therapeutic use of genes, genetic elements, and/or cells that have the capacity to enhance athletic performance

- **GENE THERAPY**

- **muscular**

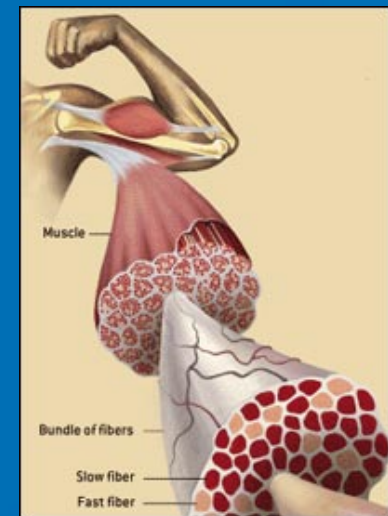
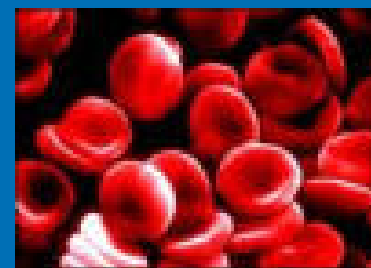
- increase muscle mass
 - IGF-1, myostatin

- **anaemia**

- increase oxygen delivery
 - EPO, VEGF

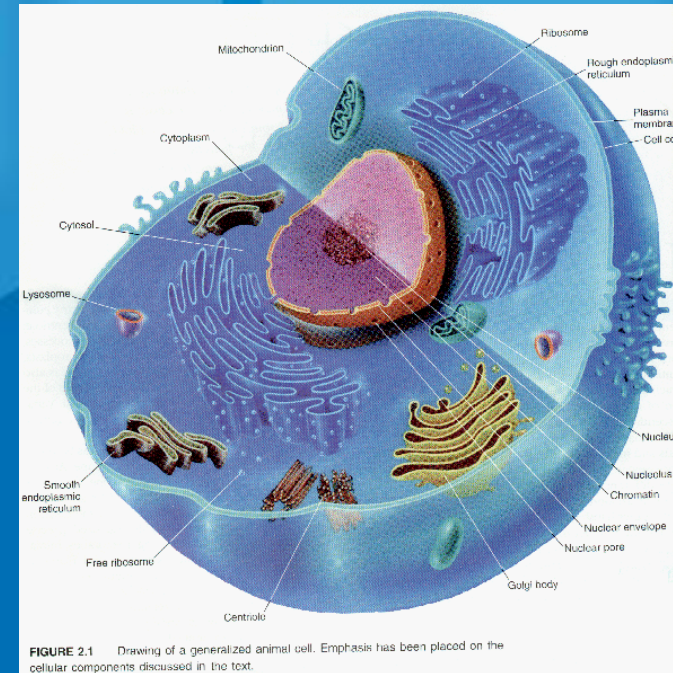
- **pain relief**

- endorphins / encephalins



Alternative testing strategy: Surrogate marker approach (biomarkers)

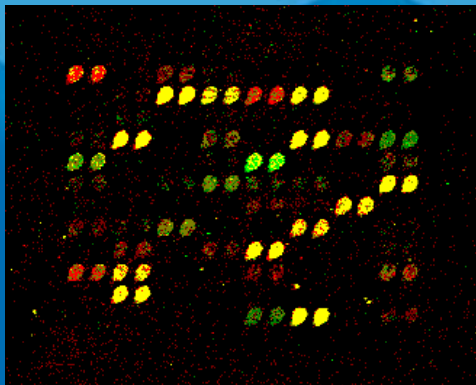
- Cell, tissue, organ, organism
 - Complete ensemble of biomolecules
 - Reflects influences of the environment
- Introduce exogenous substance (e.g. drug, gene)
 - Change in the composition of the biomolecule ensemble
- Biomarkers
 - Transcripts (mRNA)
 - Proteins
 - Metabolites (any other biomolecules)



Transcriptomics vs Proteomics

- **Transcripts (mRNA)**

- Cellular material
 - White blood cells
 - Urine epithelial cells
- Gene expression information
- Complementary to proteomics

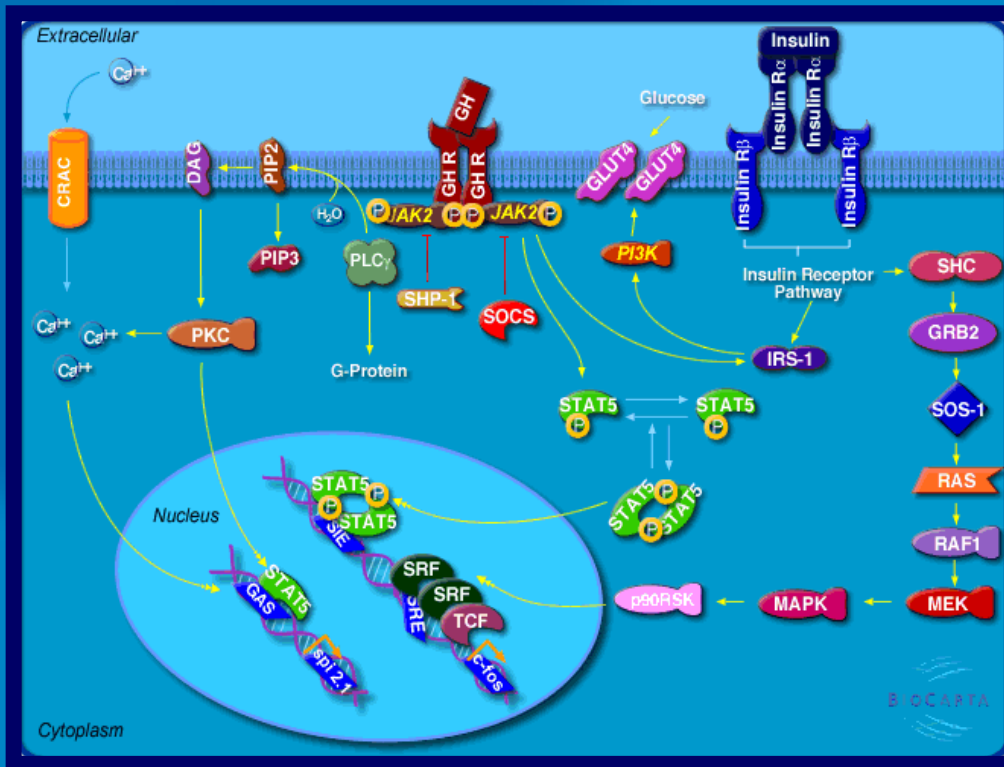


- **Proteins**

- Serum/plasma
- Soluble proteins
- True reflection of system e.g. PTM
- Simpler assay



Surrogate marker approach



Assays based on:

- effect of drug
- NOT the drug itself
- Indirect markers of drug abuse

Challenge: distinguish between samples collected from subjects +/- treatment

Screening approaches

1 Pattern recognition (uncharacterised markers)

- **Transcriptomics**
 - Microarrays, PCA, PC-DA
- **Proteomics**
 - gels, MS, ANNs – WADA grant

2 Biomarker assays (characterised markers)

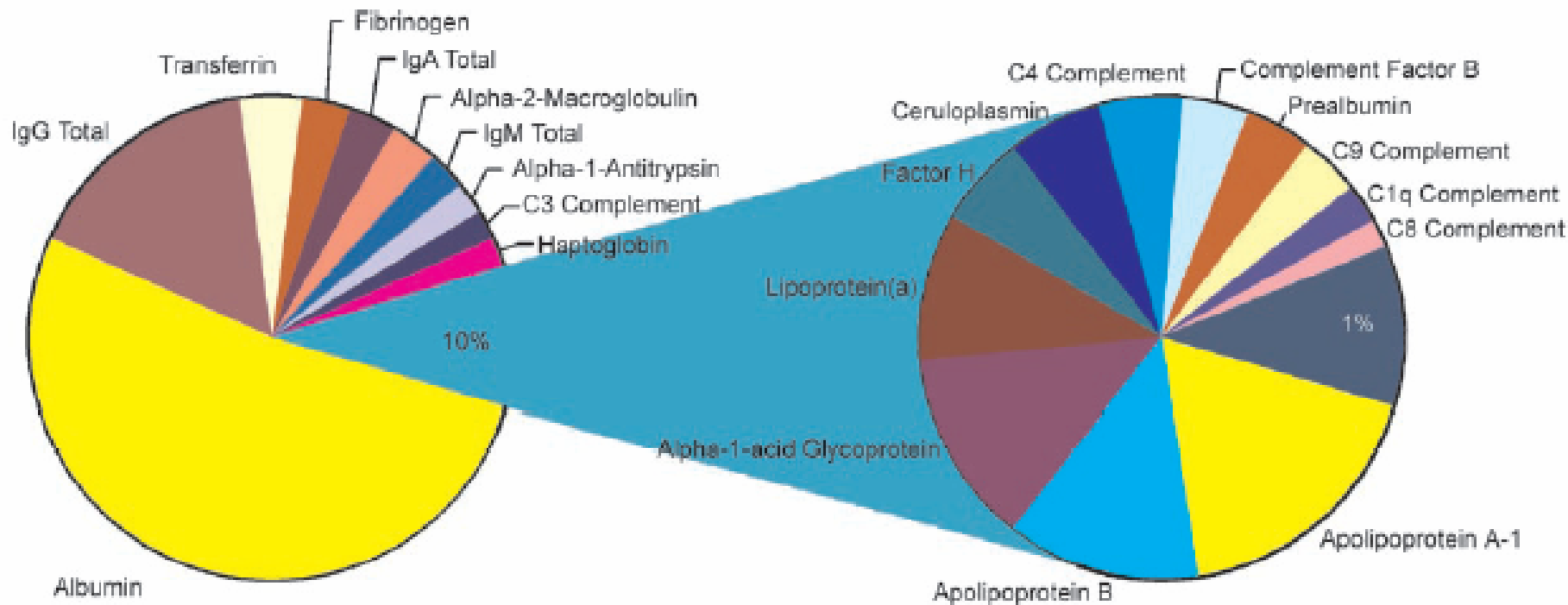
- Transcriptomics, proteomics
- Characterise proteins
- Develop panel assays (multiplexing)

1 Pattern recognition

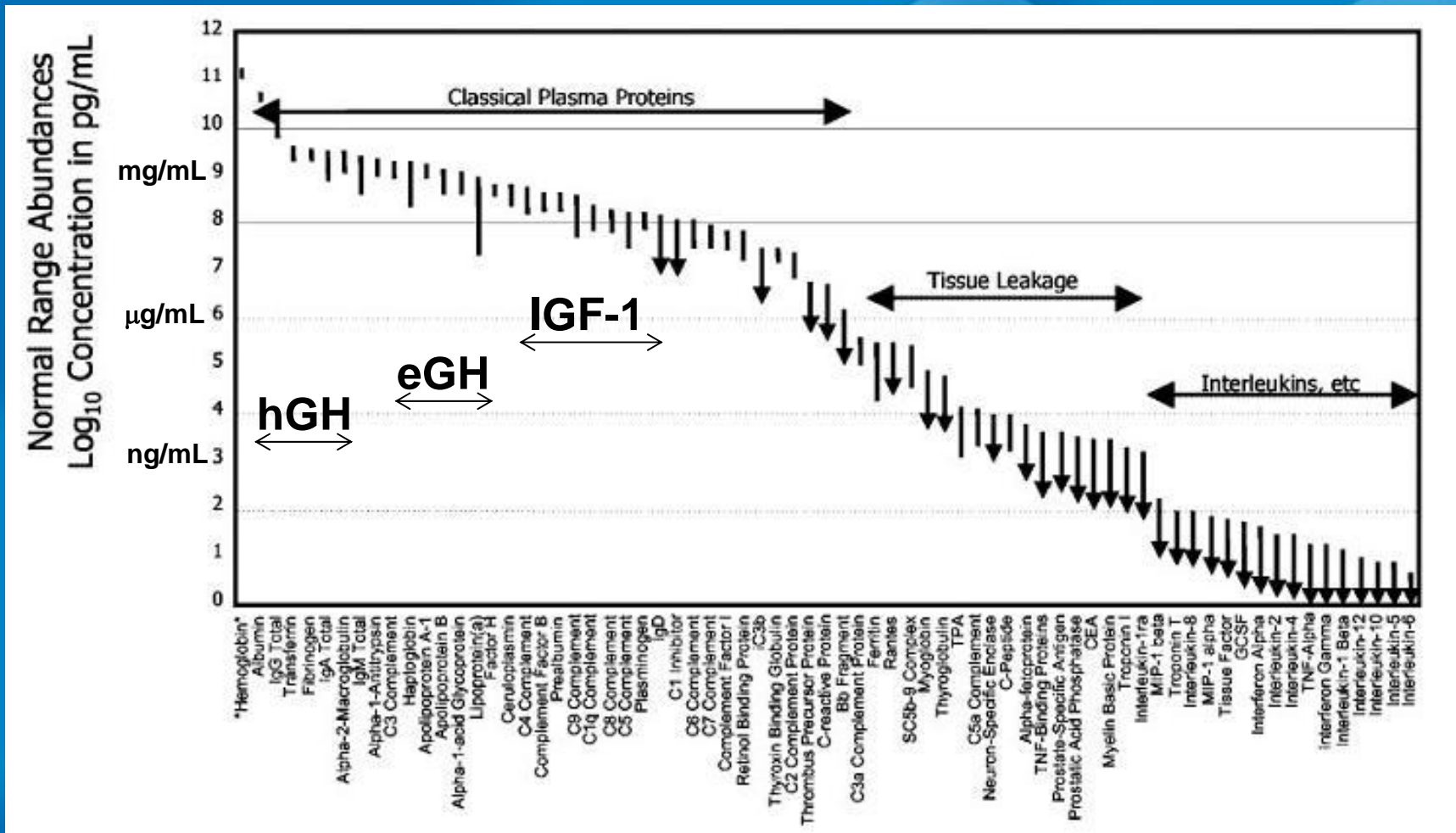
Sample preparation is key

Proteins in serum

(n=10,000, 99% from 22 proteins, 1% = 9978)



Conc of proteins and peptides in plasma

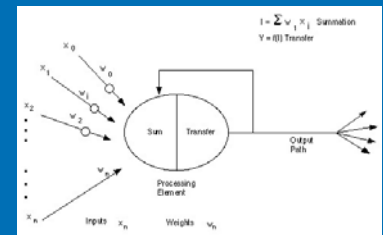
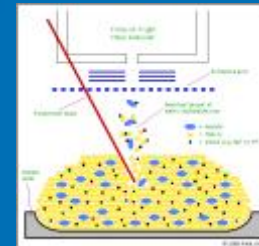


Anderson *et al* (2002) Molecular cell proteomics

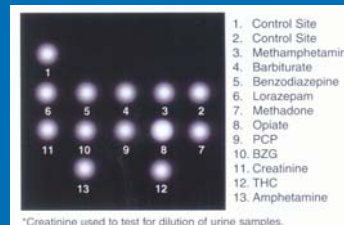
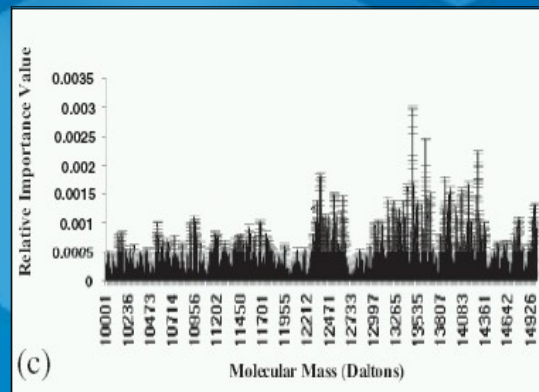
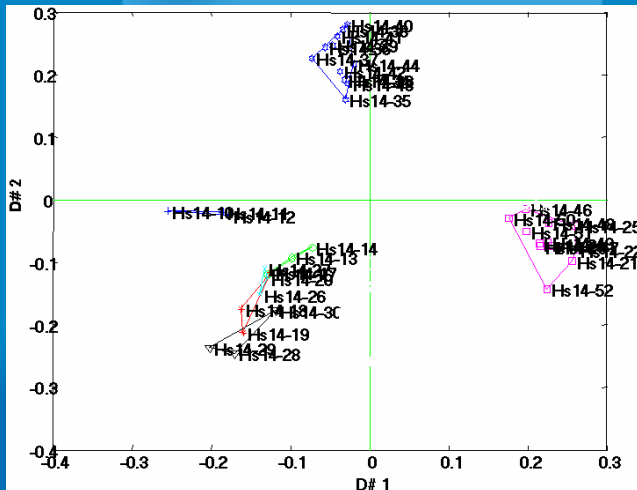
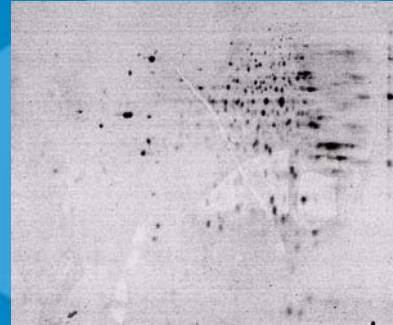
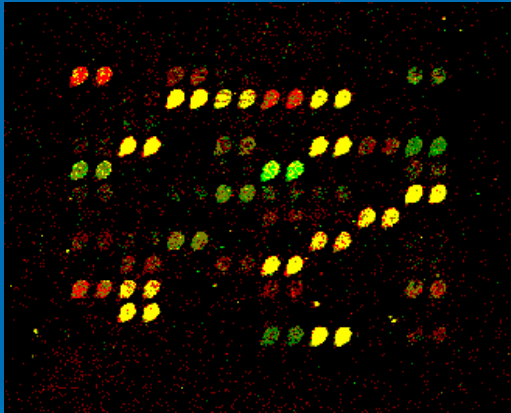


Pattern recognition - Proteomics

- Treated and Untreated subjects
 - + / - drug (gene)
 - Collect serum, blood cells, muscle
- Sample analysis
 - Protein/peptide extraction
 - MALDI / nano LC-MS
- Data analysis
 - Apply pattern recognition ANNs



2 Development of tests for biomarkers (up- or down-regulated)



Advantages of surrogate marker approach

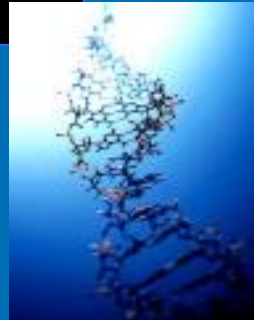
- **Ability to control abuse**

- New Pharma drugs
- Genetic modification
- Designer drugs
- Proteins/peptides



- **Future-proofing**

- Detection of effect of drug/agent
- NOT presence of agent



- **Change future of sports drug testing**



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