IMPLEMENTATION OF PREDICTIVE MODELS BASED ON METABOLOMICS-DERIVED BIOMARKERS

Gaud DERVILLY-PINEL

S. CHÉREAU, A. GICQUIAU,
S. PRÉVOST, F. MONTEAU, B. LE BIZEC

Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA)
USC INRA 1329, Oniris, LUNAM Université
BP 50707, 44307 Nantes Cedex 3, France - www.laberca.org

Outline

I. Need for alternative screening strategies

II. Metabolomics models

III. Assessment of model performances

IV. Implementation

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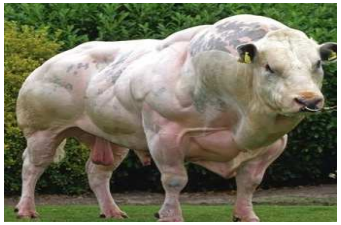
Current challenges in detecting anabolic practices in cattle

DESIGNER DRUGS

CLASSICAL APPROACH

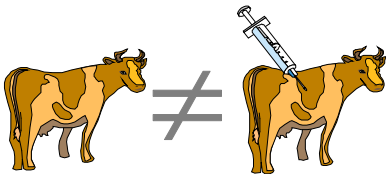
TARGETED MEASUREMENTS

EMERGING STRATEGIES

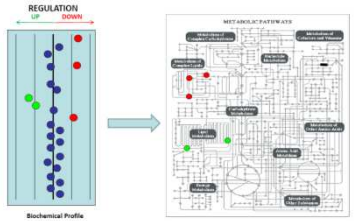


EFFECT BASED MEASUREMENTS

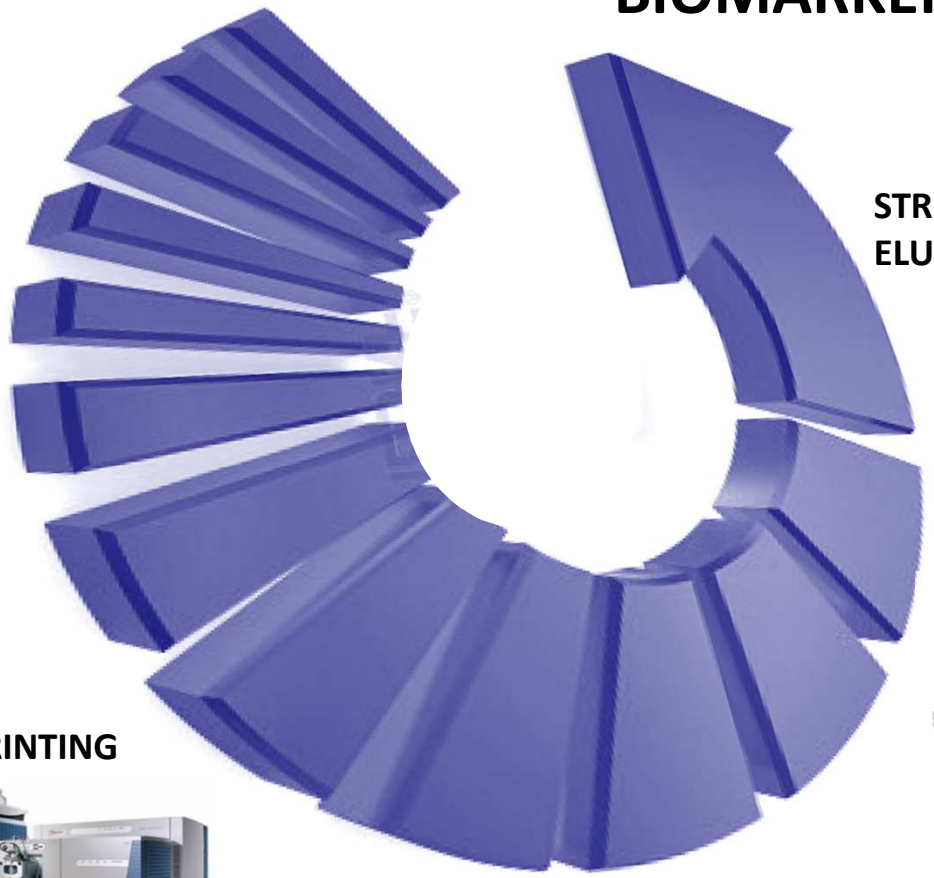
Pinel et al.TRAC 2010; 29(11), 1269.
Dervilly-Pinel et al., Drug Testing & Analysis, 2012; 4(1):1-11



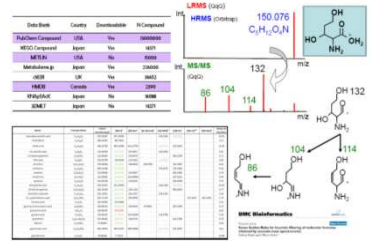
CANDIDATE BIOMARKERS



EXPERIMENT



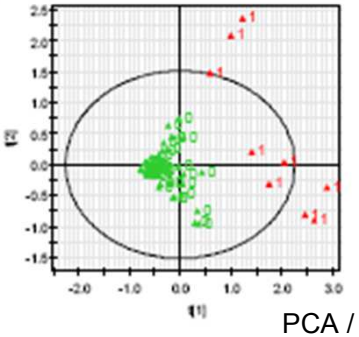
STRUCTURAL ELUCIDATION



SAMPLE PREPARATION

Urine

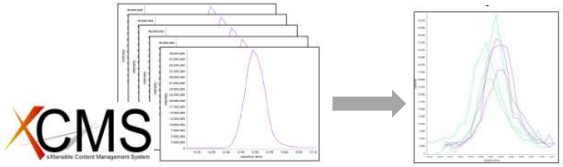
DATA ANALYSIS

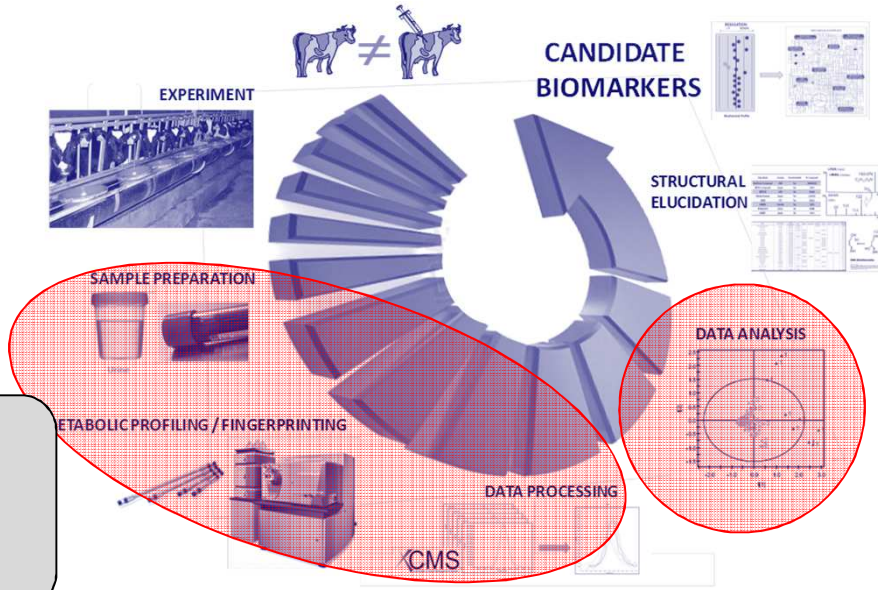


PCA / OPLS

FINGERPRINTING

DATA PROCESSING



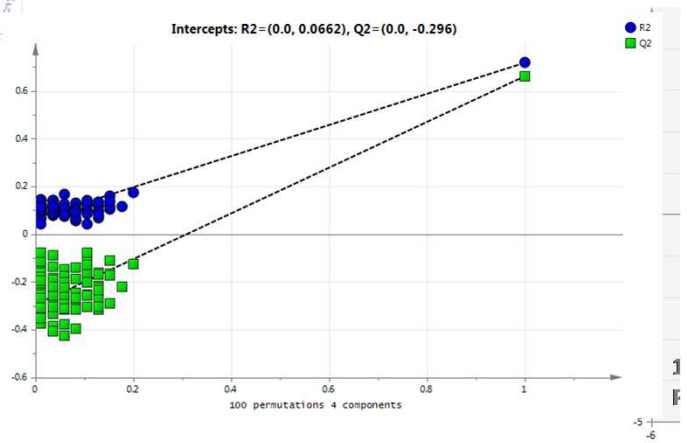


STATISTICAL VALIDATION

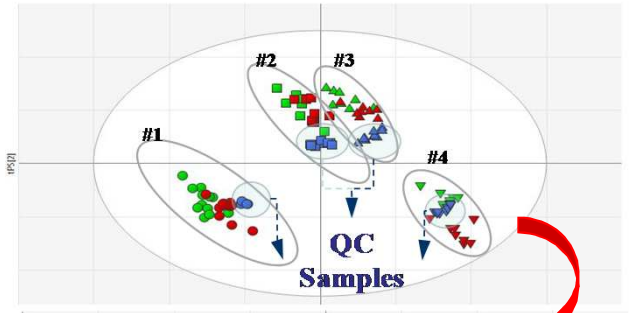
ANALYTICAL VALIDATION

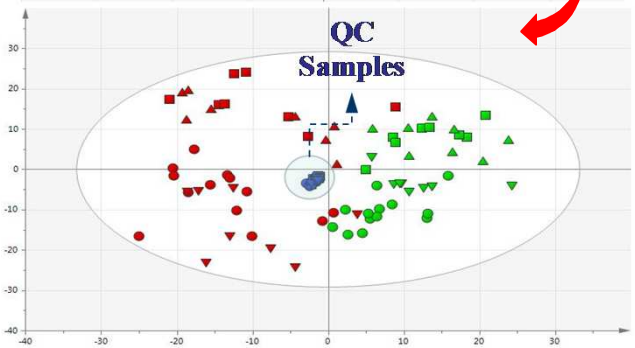
- Internal standards (*Rt, I, Δppm*)
- QC samples (*Removal of between-batch offsets and drifts*)
- Peaks selection (*CV< 30%*)

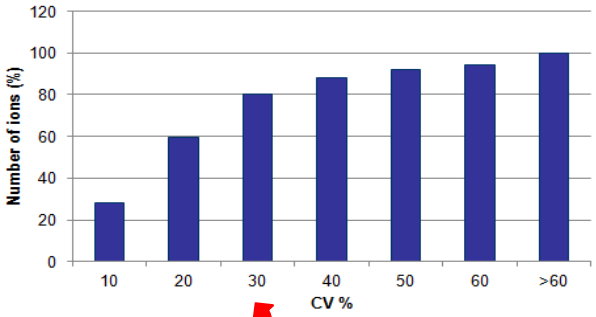
Permutation test

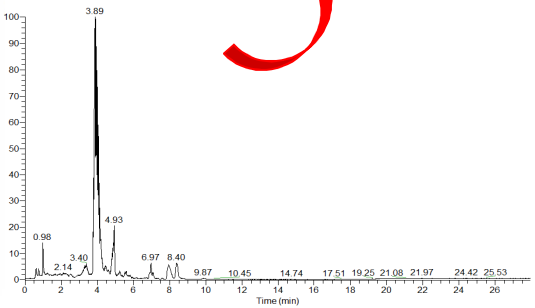


Permutation Test shows that R2(Y) and Q2(Y) values decrease upon 100 permutations

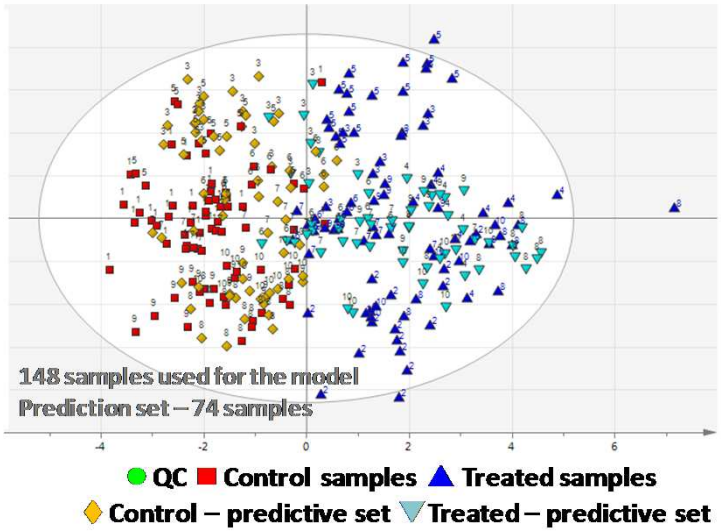








Cross-validation



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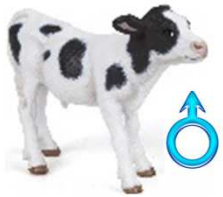
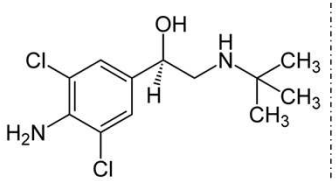
Experiment 1

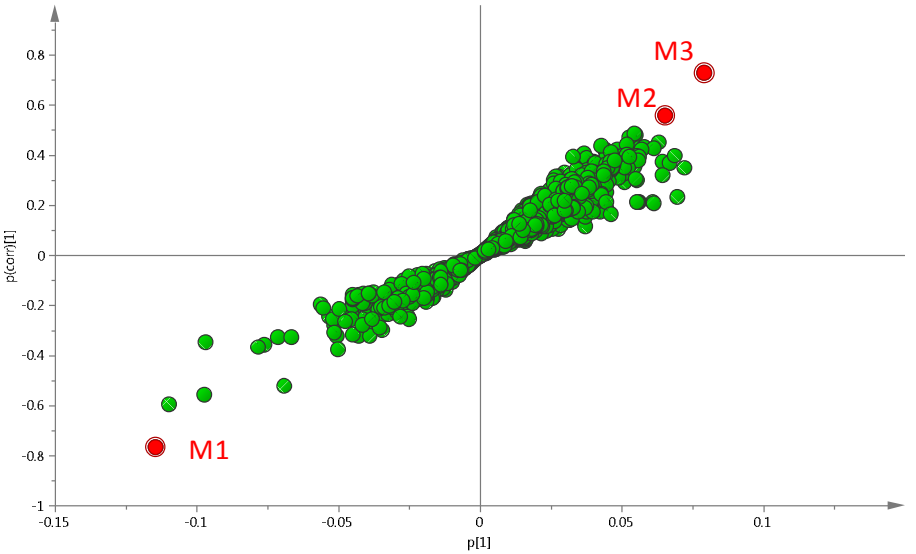


18 calves
Holstein
80 days old

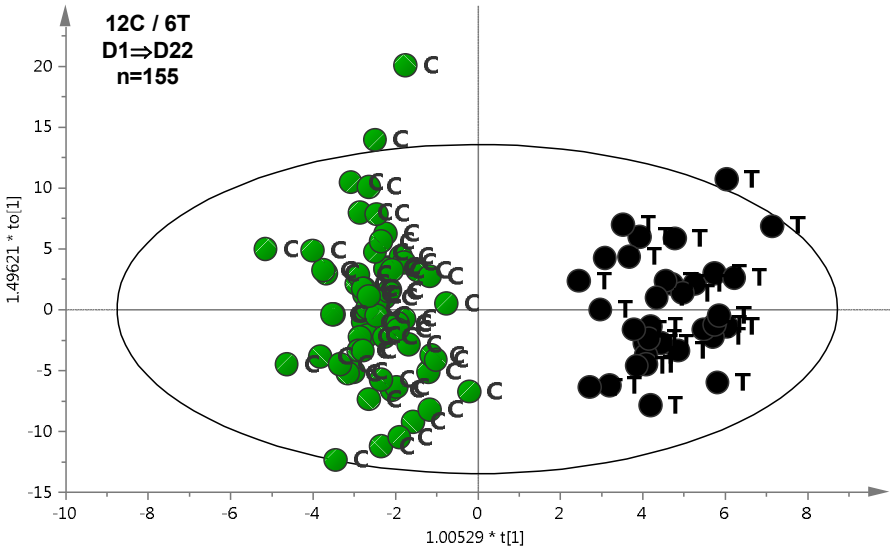


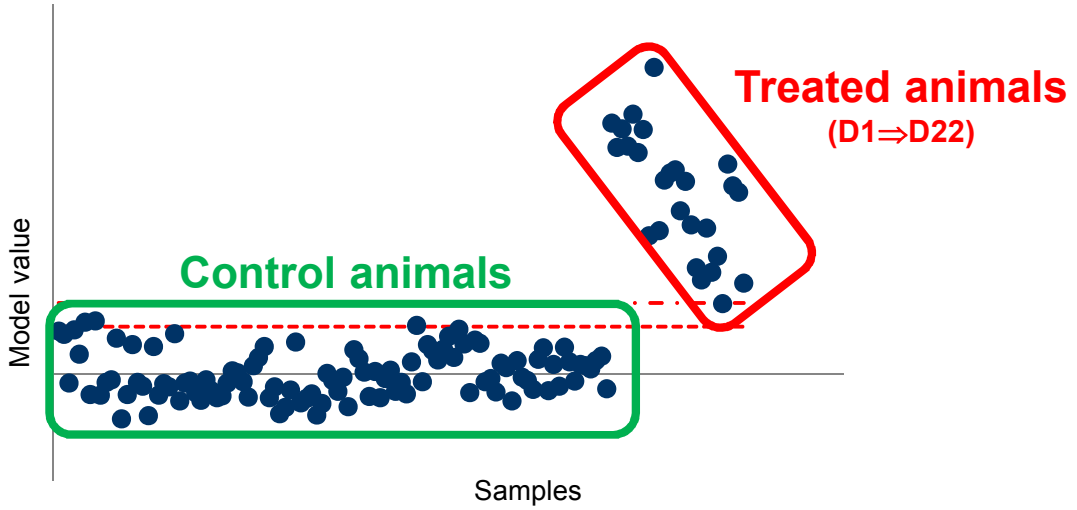
Clenbuterol

21 days
per os
10 µg/kg bw

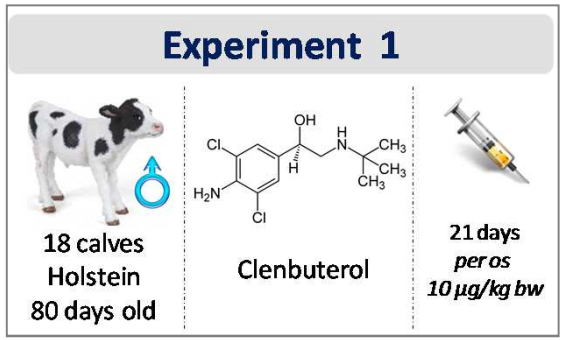


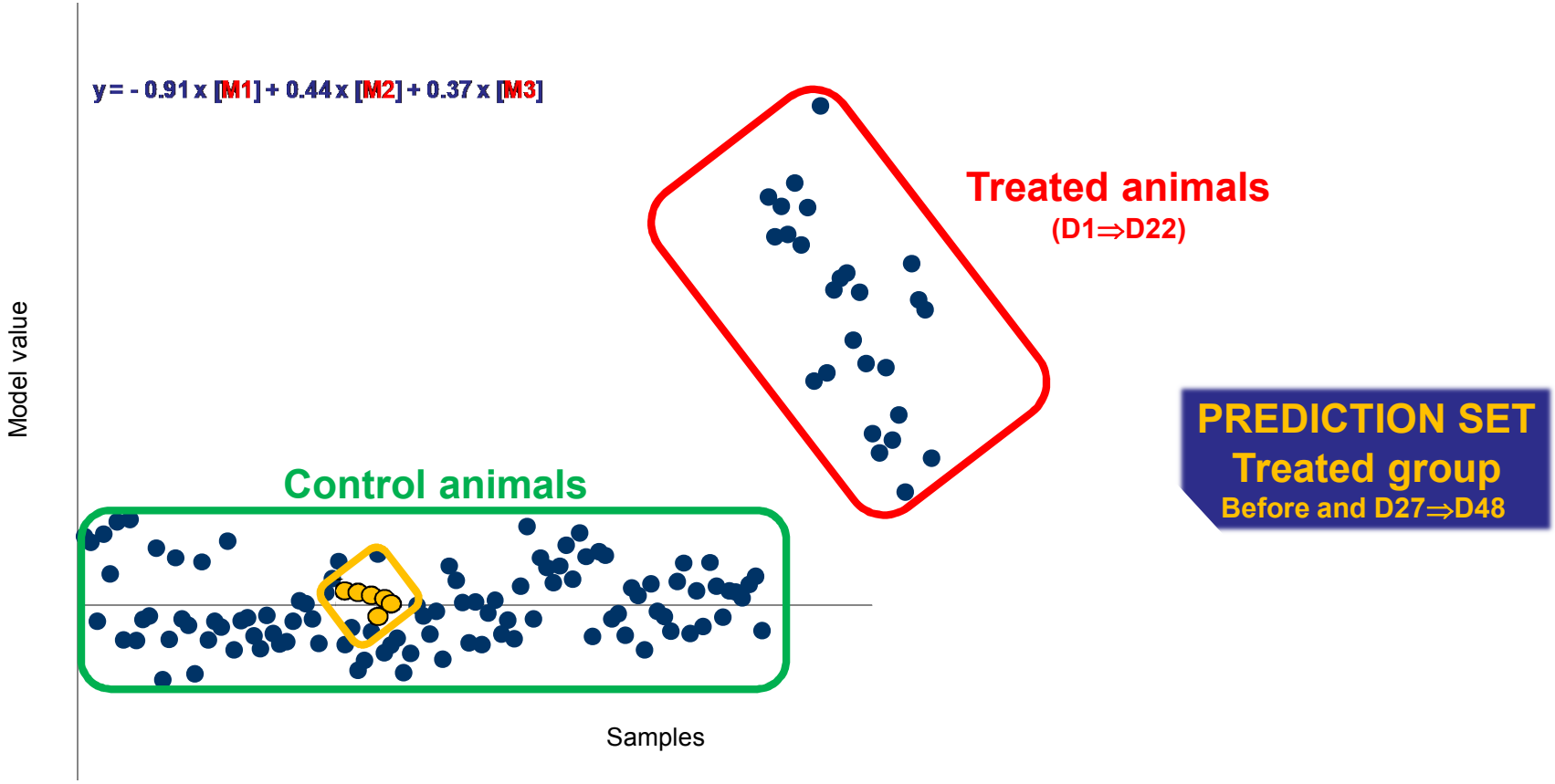
y = - 0.91 x [M1] + 0.44 x [M2] + 0.37 x [M3]





Dervilly-Pinel et al. Metabolomics 2014





Dervilly-Pinel et al. Metabolomics 2014

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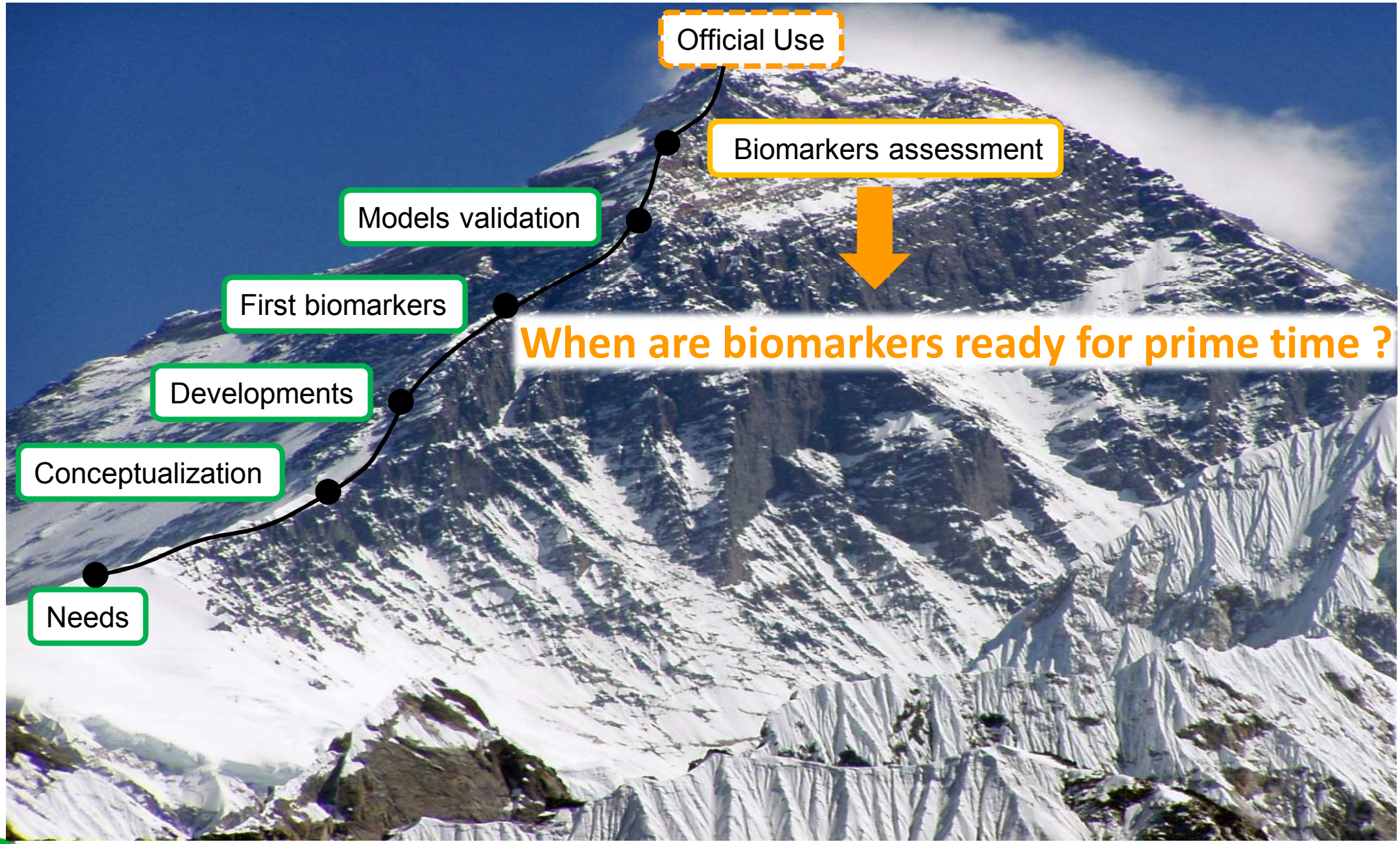

Official Use
Biomarkers assessment
Models validation
First biomarkers
Developments
Conceptualization
Needs
When are biomarkers ready for prime time ?

❑ SELECTIVITY

- Different drugs / same family
- Other growth promoters (steroids, GH, ...)
- Veterinary drugs (antibiotics, ...)

❑ SENSITIVITIES

- Low dose treatments
- Detection time window
- Analytical performances

❑ ROBUSTNESS

- Large scale testing
- Within / Ext. reproducibility

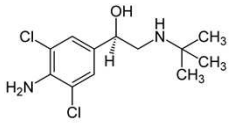
❑ PERFORMANCES

(false pos./neg. results)

❑ REGULATORY ISSUE

Experiment 2

17 calves
Holstein
80 days old



Clenbuterol

6 days
per os
5 µg/kg bw

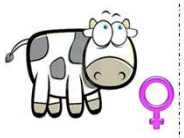
Experiment 3

3 calves
Holstein
80 days old

Low dose cocktail
- Mabuterol
- Clenbuterol
- Cimaterol
- Zilpaterol
- Ractopamine

5 days
per os
1 µg/kg bw (each)

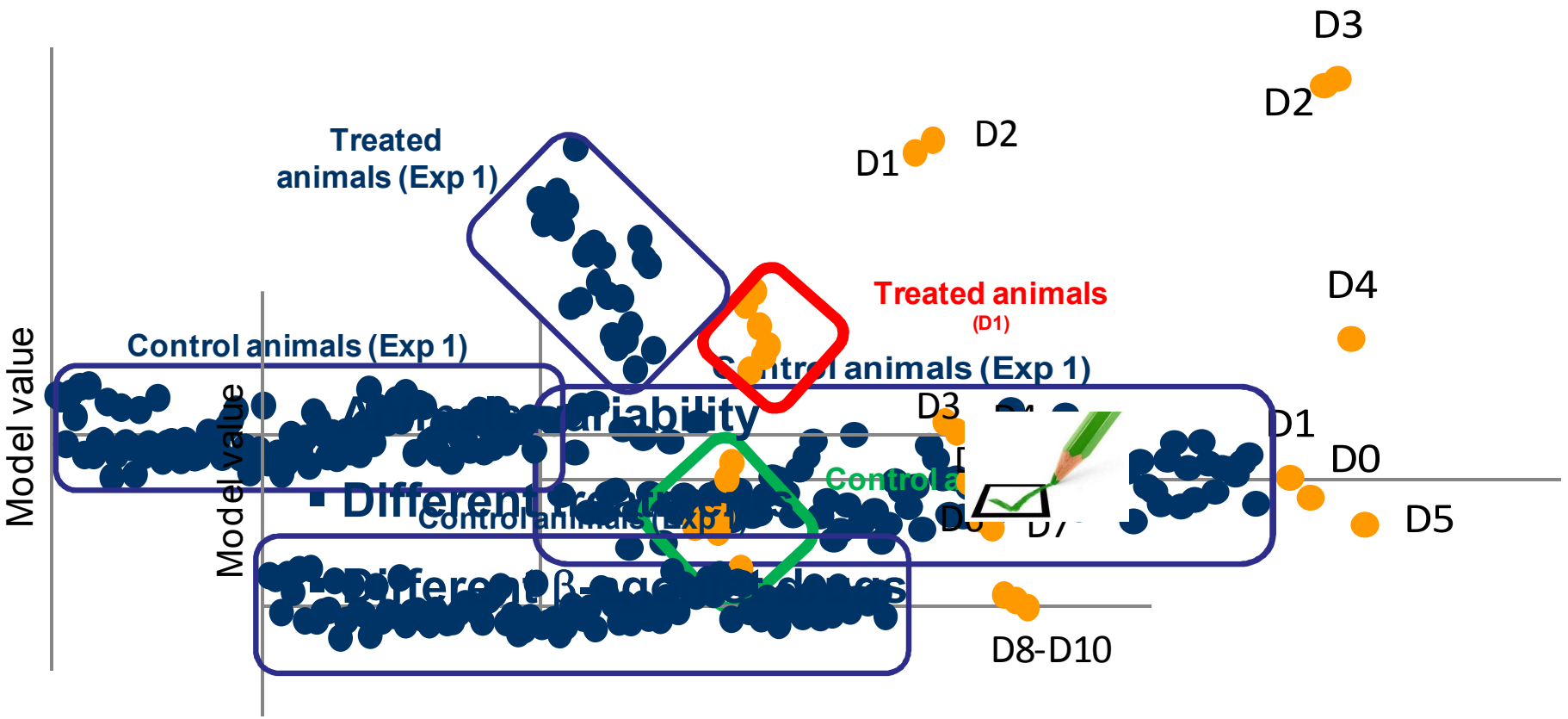
Experiment 4



3 cows
Holstein
>36 month old

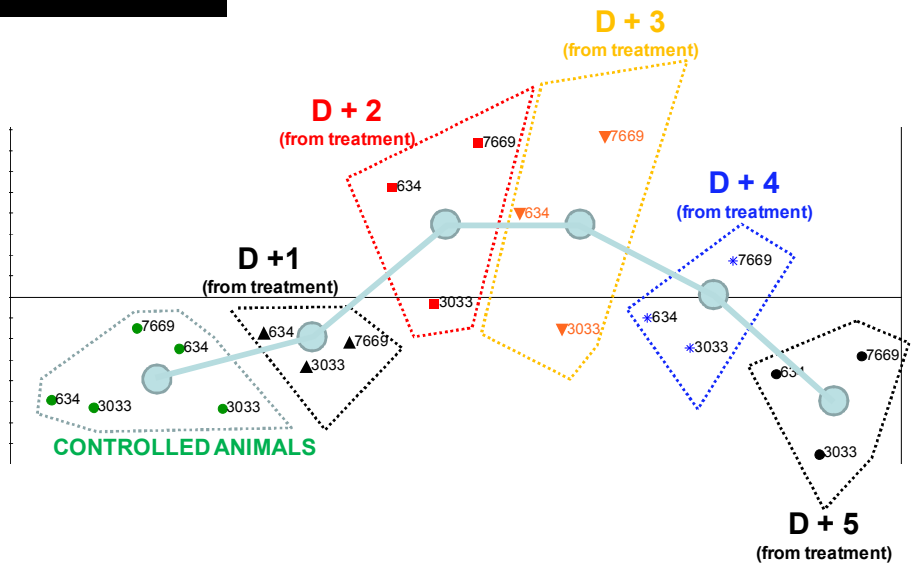
- Clenbuterol
- Ractopamine
- Cocktail
  Clenbu + Racto

1 day
per os
1-200 µg/kg bw

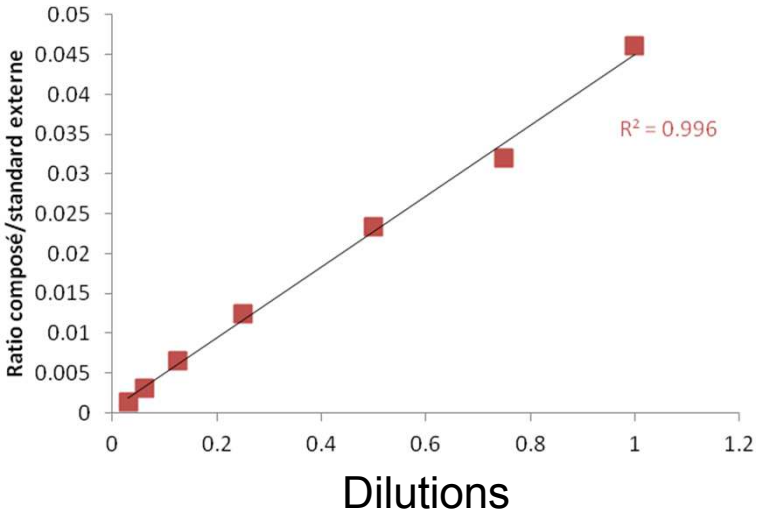


Dervilly-Pinel et al. Metabolomics 2014

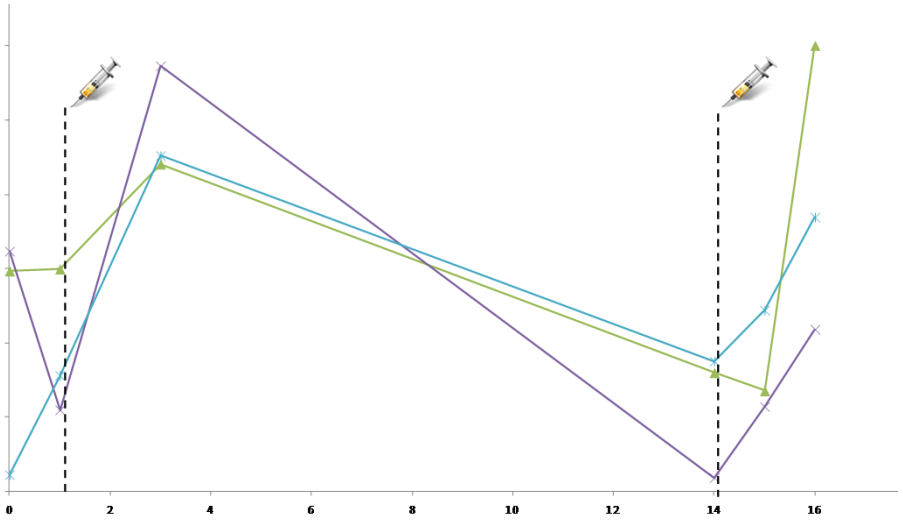
TIME WINDOW



ANALYTICAL SENSITIVITY



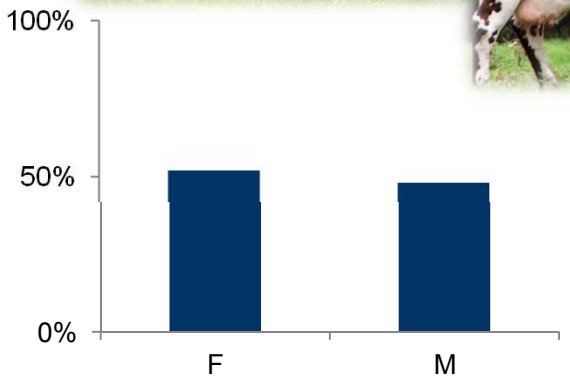
DIFFERENT ANABOLIC TREATMENTS



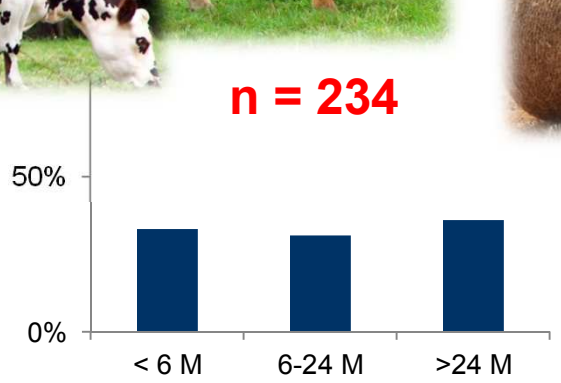
n = 3

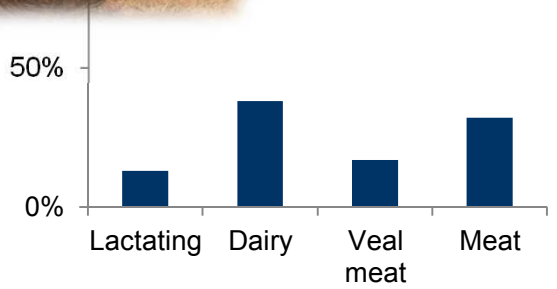
19-NTD+E2Bz

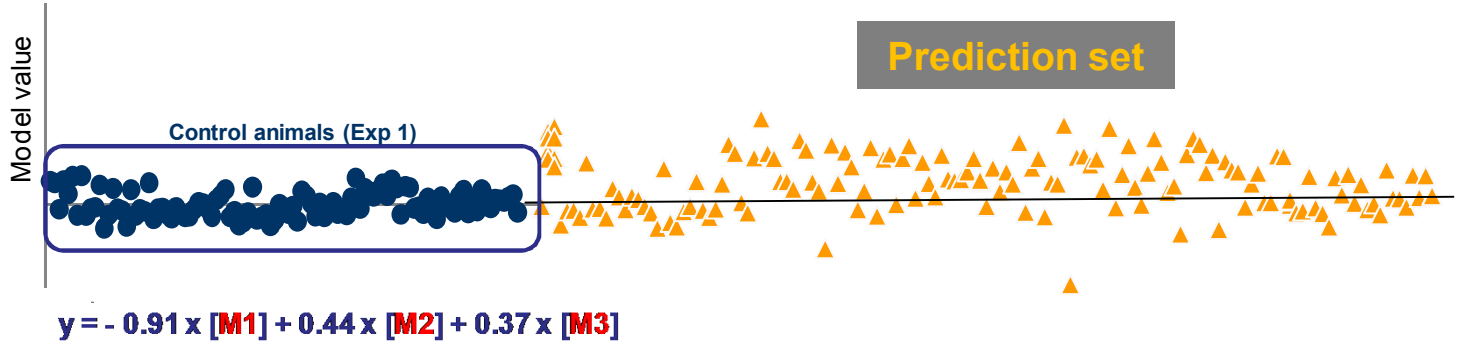
Large Scale testing - Robustness



n = 234





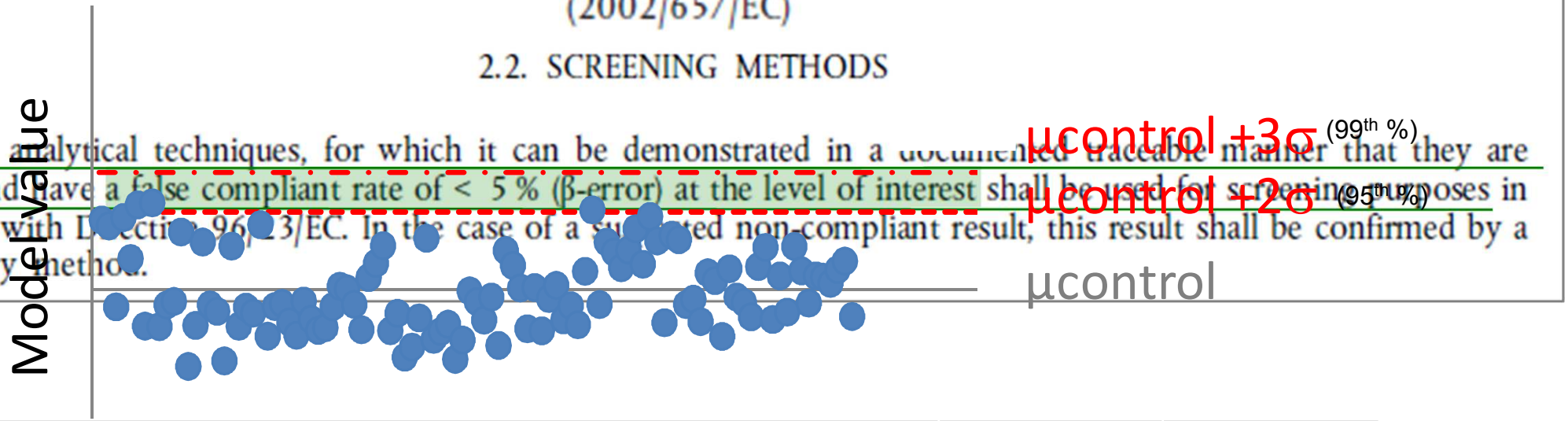


COMMISSION DECISION

(2002/657/EC)

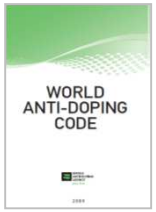
2.2. SCREENING METHODS

Only those analytical techniques, for which it can be demonstrated in a documented traceable manner that they are validated and have a false compliant rate of < 5 % (β-error) at the level of interest shall be used for screening purposes in conformity with Directive 96/23/EC. In the case of a suspected non-compliant result, this result shall be confirmed by a confirmatory method.



			95th perc.	99th perc.
Whole population	n=534	% False Compliant	0.2	1.3
		% False non Compliant	20	15

Human



WORLD ANTI-DOPING AGENCY

Article 2.1.2

Sufficient proof of an anti-doping rule violation is established either by the presence of a prohibited substance, its metabolites or markers [...]

WADA's Executive Committee approved WADA's Athlete Biological Passport Operating Guidelines on December 1, 2009.

Horses

Article 6, §11

[...] The finding of any scientific indicator of administration or other exposure to a prohibited substance is also equivalent to the finding of the substance [...]

New version approved in April 2009 "International Agreement on Breeding, Racing and Wagering"

Live animals, animal products, their feed

No L 125/10 EN Official Journal of the European Communities

COUNCIL DIRECTIVE 96/23/EC

of 29 April 1996

on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC

Article 1

This Directive lays down measures to monitor the substances and groups of residues listed in Annex I.

Article 2

'residue' shall mean a residue of substances having a pharmacological action, of their metabolites and of other substances transmitted to animal products and likely to be harmful to human health;

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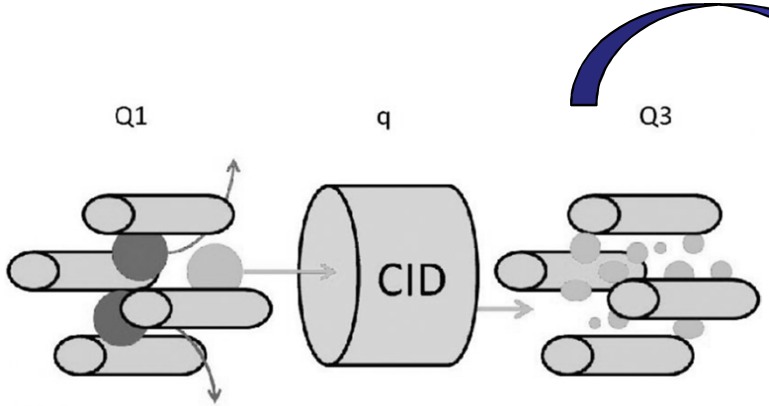
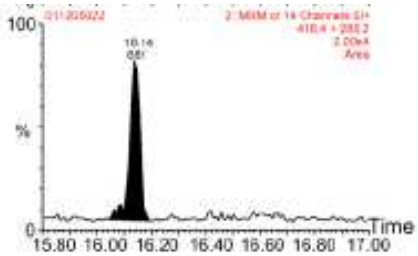
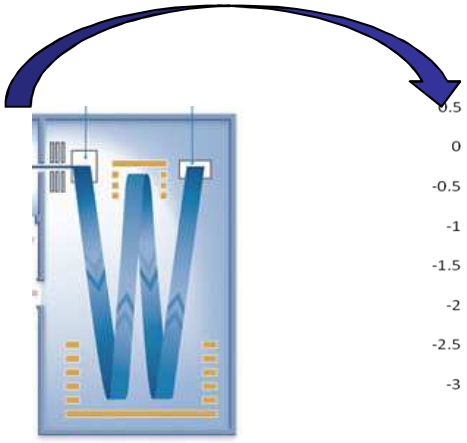
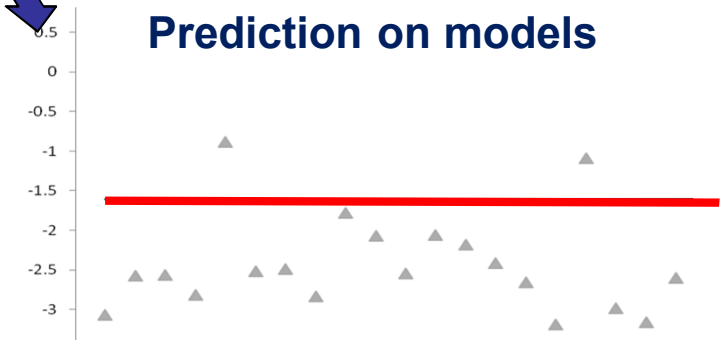
IDENTIFIED & LIMITED NUMBER OF GENERIC BIOMARKERS





SET OF NON IDENTIFIED BIOMARKERS

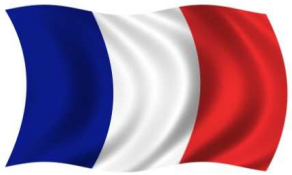




- **SCREENING REQUIREMENTS ?**

- **IS IT IMPLEMENTABLE ON A LARGE SCALE ?**

Strategy added since 2013 in the French National Monitoring Plan



- **IS IT ACCREDITABLE ?**

- **IS THE STRATEGY TRANSFERABLE TO SMEs ?**

EU FP7 project DeTECH 21 (2013-2015)

CAPACITIES

ANSWERS HUMAN HEALTH/FOOD SAFETY ISSUES

ACKNOWLEDGEMENTS

http://www.laberca.org
http://www.saraf-educ.org

Région BRETAGNE

RÉGION PAYS DE LA LOIRE

SIXTH FRAMEWORK PROGRAMME

AND YOU FOR YOUR ATTENTION !

École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation — Nantes Atlantique

Santé et alimentation au cœur de la vie

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BP 50707, 44307 Nantes Cedex 3, France - www.laberca.org