



*Genotype **plus** Environment*  
*Integration for a more sustainable dairy production system*

# Potential of milk MIR spectra to obtain new health phenotypes for dairy cows in the GplusE project

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**[www.gpluse.eu](http://www.gpluse.eu)**



- Breeding needs to be more and more efficient



- Purpose : Realize a Genome Wide Association Study (GWAS) for dairy cows  
→ Genotypes // Phenotypes of interest





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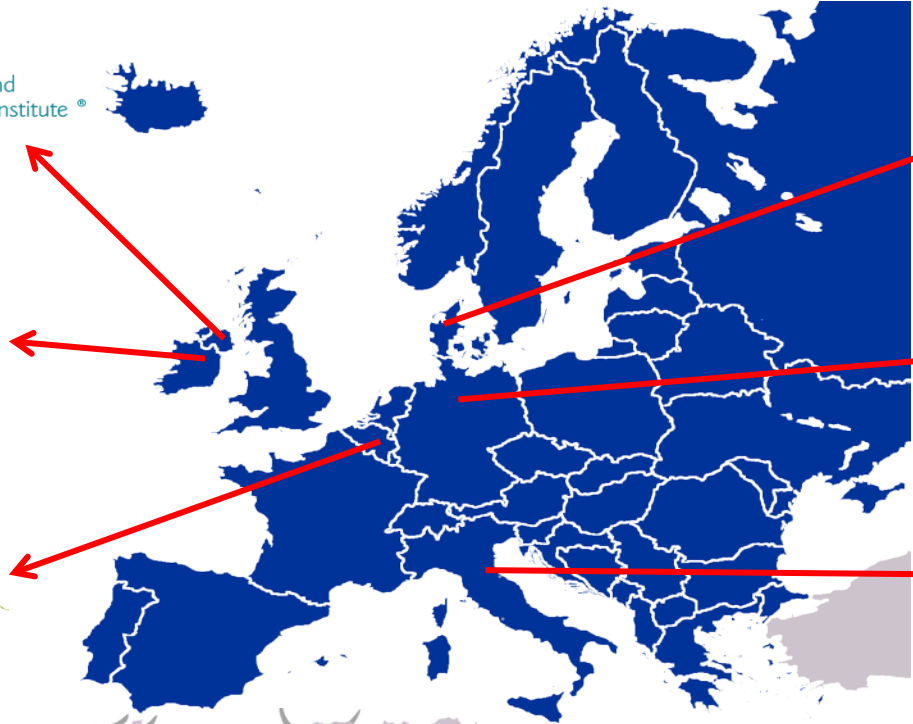




- 1<sup>st</sup> step : collect phenotypic data around calving linked with biomarkers to
    - create a reference database,
    - identify the link between biomarkers and phenotypic information,
    - **develop tools to obtain the information quickly/easily/in routine.**
    - use them for the next steps of the project :
- more than 10 000 cows will be genotyped and milk samples will be taken
- link between genetic information and predicted phenotypes



# Creation of the reference database



# Creation of the reference database

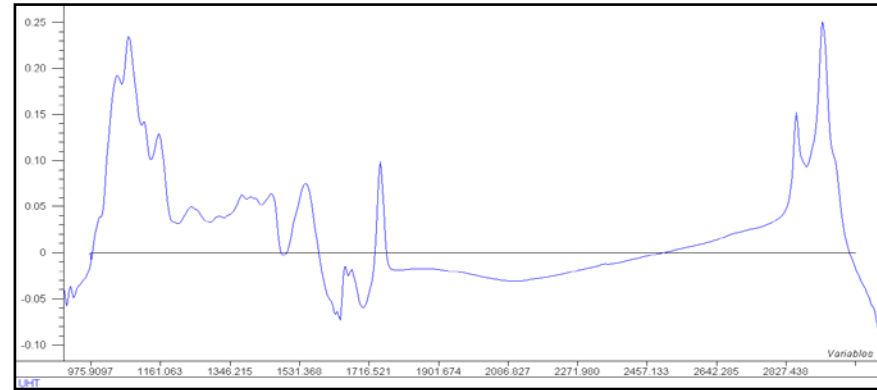
- **Phenotypes of interest** : RFI, Energy balance, ketosis, lameness, hock, mastitis, metritis, loss of weight after calving, etc.
- **Biomarkers** : milk and blood metabolites, milk glycanes, **milk MIR spectra**



# Milk Mid Infrared Spectrometry



Each MIR  $\lambda$



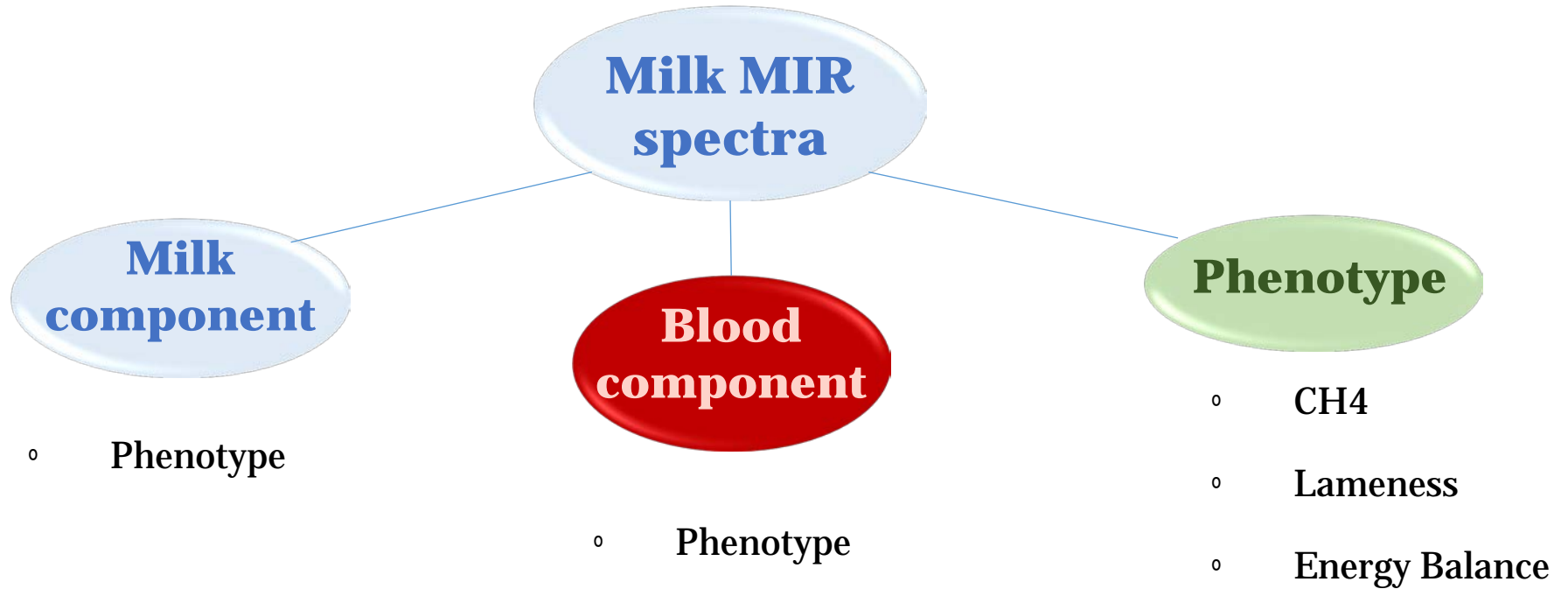
# Milk Mid Infrared Spectrometry

- Key interests of milk MIR spectra :
  - Already collected in routine
  - Cheap
  - Quick
  - Reflect the milk composition → could be a “mirror” of the health status of cow
  - Standardized spectra → equations of prediction can be used in every milk recording lab involved in standardization

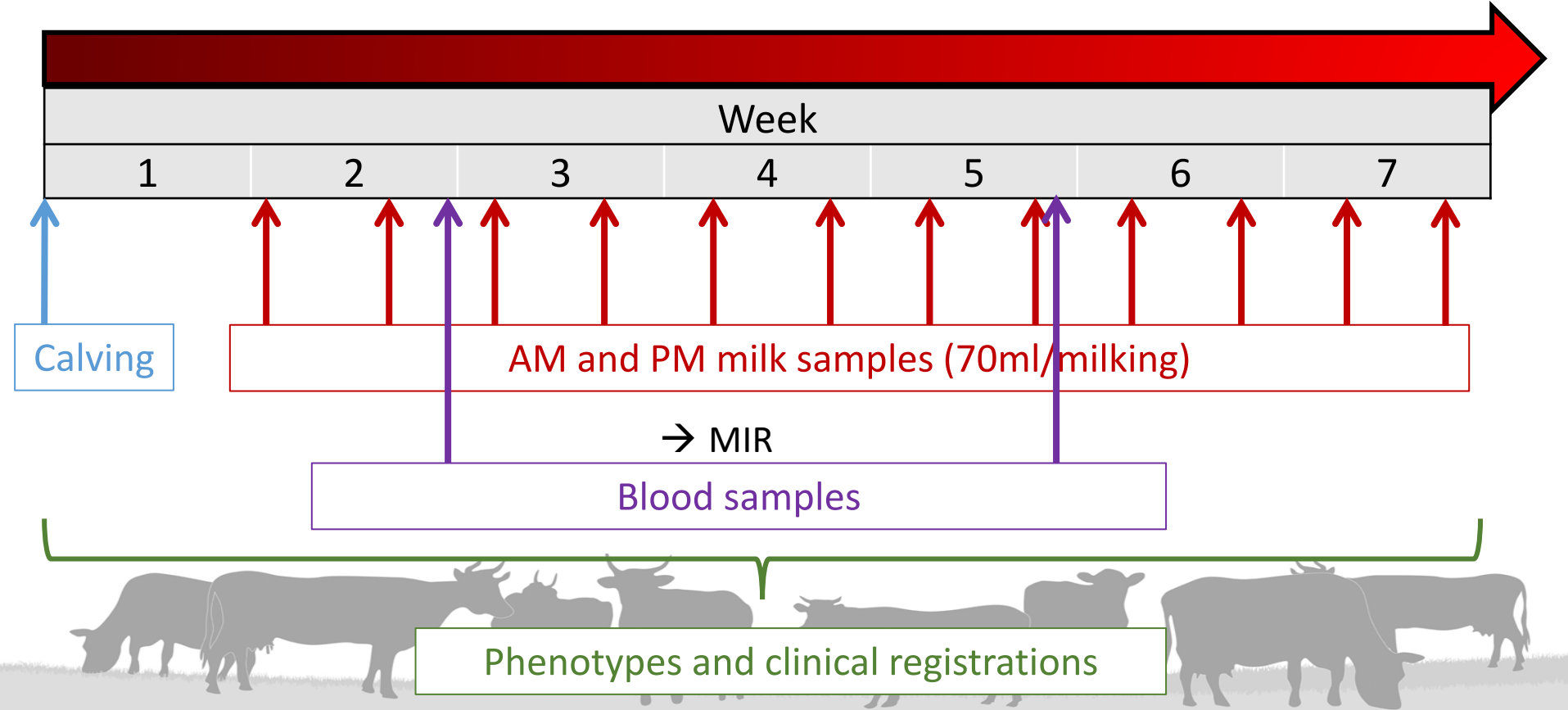




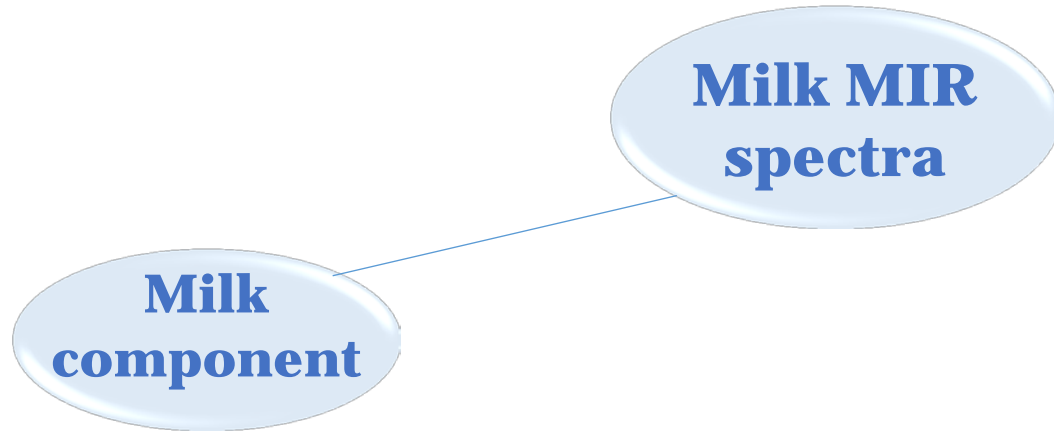
# Milk Mid Infrared Spectrometry in GplusE



# Sampling protocol



# Milk Mid Infrared Spectrometry in GplusE



- Phenotype

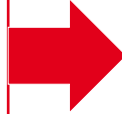


# Prediction of milk component

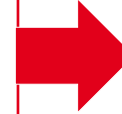
**NAGase**  
(*N*-acetyl- $\beta$ -D-glucosaminidase)

=

**The tissue damage marker enzyme**



**In milk**  
→  
**indicator of mastitis**



**4-6  $\mu\text{mol product / min x L}$**

**= alert for potential sub clinical mastitis**

(T. Larsen, 2016,  
personal communication)



# Prediction of milk component

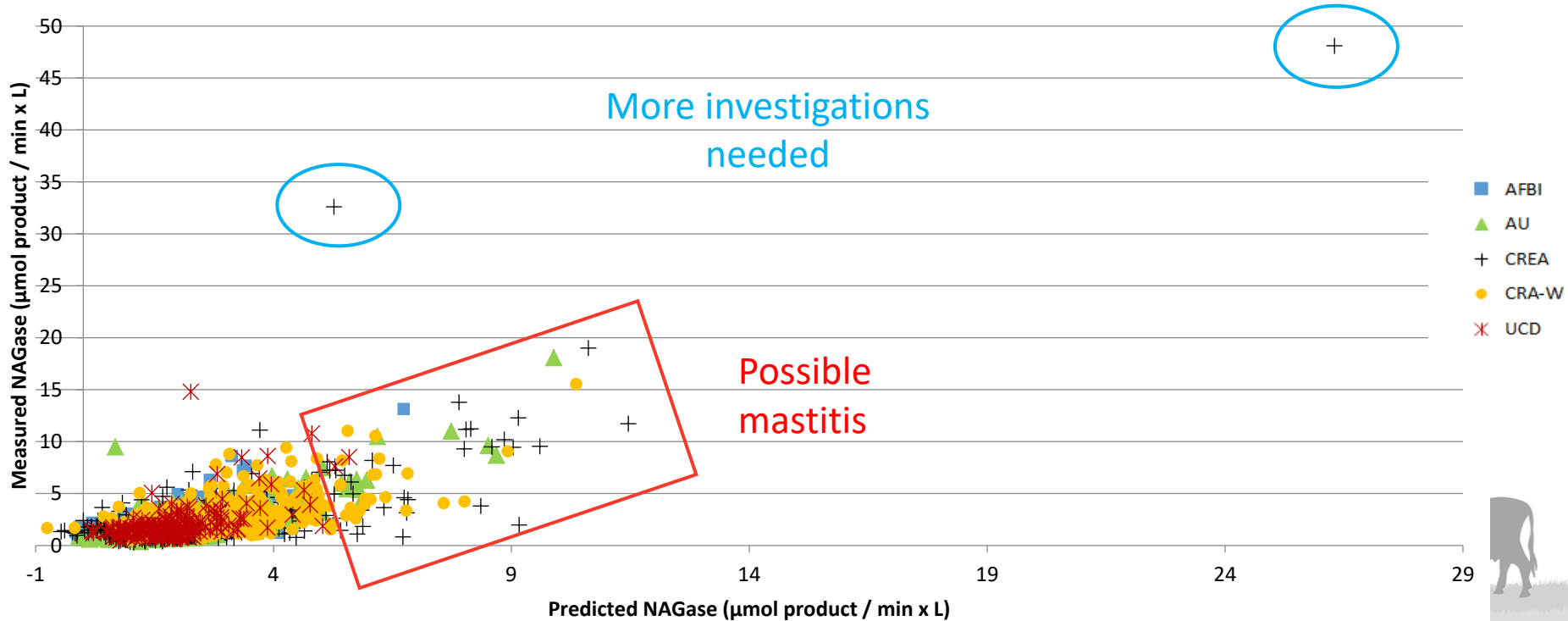
- NAGase
- Milk analyses// closest milk MIR spectra (day  $\pm$  2)

Constituent	AFBI (UK)	CRA-W (BE)	CREA (ITA)	UCD (IRL)	AU (DK)	TOTAL
NAGase	682	335	542	194	375	<b>2129</b>

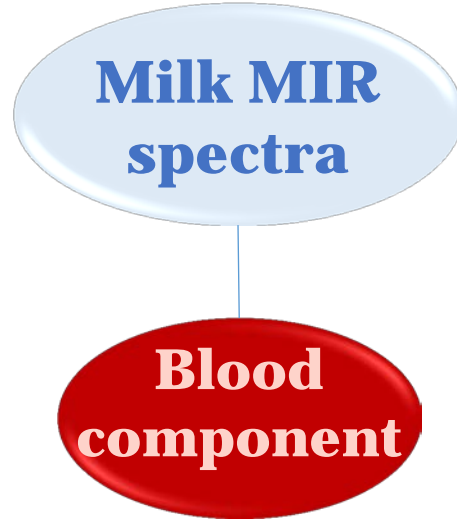


# Prediction of milk component

Constituent	N	Mean	SD	R <sup>2</sup> c	R <sup>2</sup> cv	SEC	SECv
NAGase	2129	2.12	2.05	0.50	0.43	1.45	1.55



# Prediction of blood component



◦ Phenotype



# Prediction of blood component

In early lactation:  
energy intake < energy output

Negative energy balance

Body fat mobilisation

If excessive :

⇒ imbalance in hepatic  
carbohydrate and fat  
metabolism

⇒ ↑ of ketone bodies in blood

= Ketosis type I

BOHB known as  
biomarker

**1.2mmol/L**  
González et al., 2011  
Duffield et al., 1977

**Between 1.2mmol/L  
and 1.4mmol/L**  
Leblanc 2010





# Prediction of blood component

- BOHB
- Blood analyses // closest milk MIR spectra (day  $\pm$  2)

Constituent	AFBI (UK)	CRA-W (BE)	CREA (ITA)	UCD (IRL)	AU (DK)	TOTAL
BOHB	121	55	87	44	66	<b>373</b>



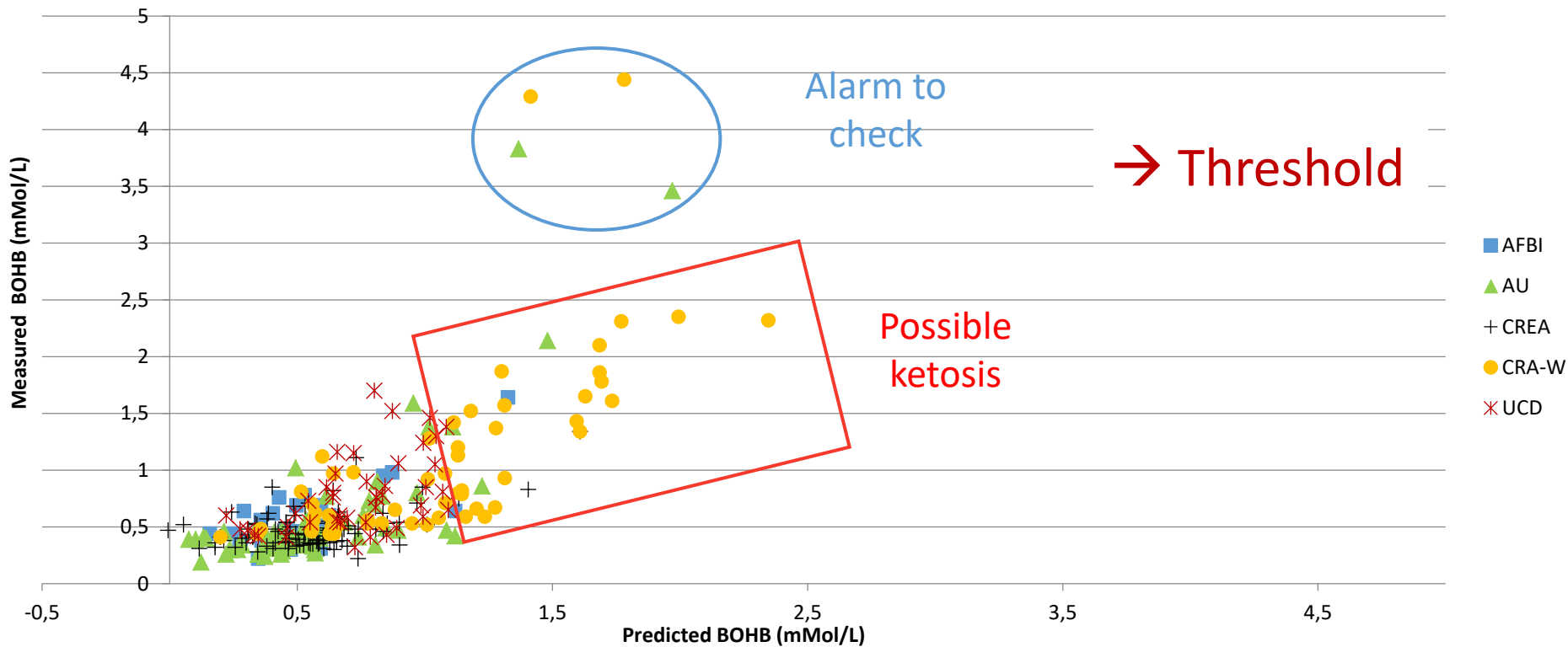
# Prediction of blood component

Constituent	N	Mean	SD	R <sup>2</sup> c	R <sup>2</sup> cv	SEC	SECV
BOHB	373	0.63	0.5	0.48	0.4	0.36	0.39



# Prediction of blood component

Constituent	N	Mean	SD	R <sup>2</sup> c	R <sup>2</sup> cv	SEC	SECv
BOHB	373	0.63	0.5	0.48	0.4	0.36	0.39



# Direct prediction of phenotypes

**Milk MIR  
spectra**

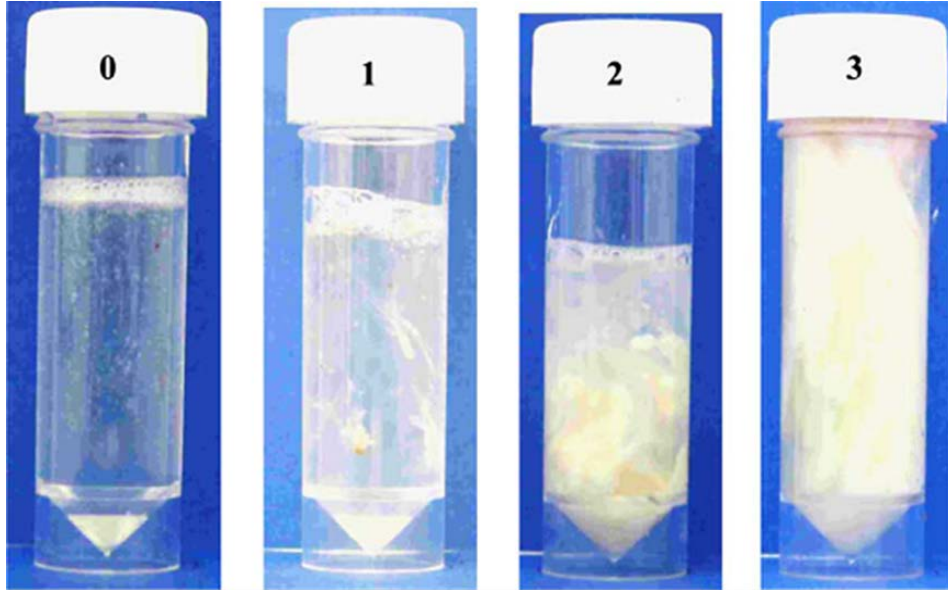
**Phenotype**

- Lameness
- Energy Balance
- CH<sub>4</sub>



# Direct prediction of phenotypes

## ◦ Uterine Discharge Appearance



### ***Appearance/coloration***

0 = clear or translucent mucus

1 = mucus containing flecks of white or white pus

2 = discharge containing  $\leq 50\%$  white or off-white mucopurulent material

3 = discharge containing  $\geq 50\%$  white or off-white purulent material, usually white or yellow, but occasionally sanguineous



# Direct prediction of phenotypes

- Uterine Discharge Appearance
- Data: 281
- Observation: J14 & J35 // MIR spectra of day  $\pm 2$

◦ Healthy: score 0

◦ UDA+: score 2, 3

◦ SVM

		Observations from experiments	
		UDA+	Healthy
MIR classification	UDA+	98	40
	Healthy	32	111
		130	151
		75%	74%



# Direct prediction of phenotypes

- Uterine Discharge Appearance

	AFBI		AU		CRA Italy		CRA-W		UCD	
	Healthy	UDA+	Healthy	UDA+	Healthy	UDA+	Healthy	UDA+	Healthy	UDA+
Predicted Healthy	30	11	13	2	25	10	16	10	12	1
Predicted UDA+	13	26	8	28	12	33	8	21	0	2
Total	43	37	21	30	37	43	24	31	12	3
Good classification	70%	70%	62%	93%	68%	77%	67%	68%	100%	67%



# Direct prediction of phenotypes

## ◦ Lameness

SCORE	CLINIC	SCORE	CLINIC
0	Movement <i>free and easy</i>	5	<i>Slightly limping</i> on one or more legs
1	Indication of <i>soreness</i>	6	<i>Limping</i> and/or <i>lameness</i> on one or more legs
2	<i>Relief</i> of leg, no visible limping	7	High degree of <i>limping</i> and/or <i>lameness</i> – leg <i>lifted</i> from the ground
3	<i>Relief</i> of outer- or inner claw, front legs <i>crossed</i>	8	Movement <i>inhibited</i> to a high degree – <i>non physiological positioning</i>
4	Hind legs <i>far apart</i> , movement <i>mildly affected</i>	9	<i>Disabled, not moving at all</i>





# Direct prediction of phenotypes

- Lameness
- Data: 201
- Observation: J14 & J35 // MIR spectra of day  $\pm 2$
- Healthy: score 0
- Lameness: score 3, 4, 5, 6, 7
- PLS-DA

		Observations from experiments	
		Healthy	Lame
MIR classification	Healthy	123	8
	Lame	44	26
		167	34
		74%	76%



# Direct prediction of phenotypes

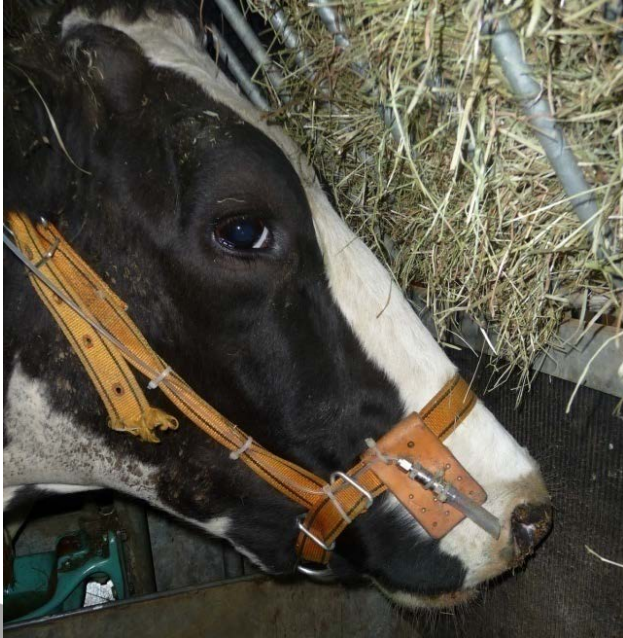
- Lameness

	AU		CRA Italy		CRA-W		UCD	
	Healthy	Lame	Healthy	Lame	Healthy	Lame	Healthy	Lame
Predicted Healthy	3	2	70	6	56	0		2
Predicted Lame	20	15	8	2	2	0	8	7
Total	23	17	78	8	58	0	8	9
Good classification	13%	88%	90%	25%	97%		0%	78%



# Direct prediction of phenotypes

- Methane (CH<sub>4</sub>)
- Existing equation (MethaMilk)



◦ (A-W)  
(gasc)  
(ETH  
(Con  
(BN)



Credit : Kathrin Buehler, UZH

SF6

Chambres

# Direct prediction of phenotypes

- Methane (CH<sub>4</sub>)

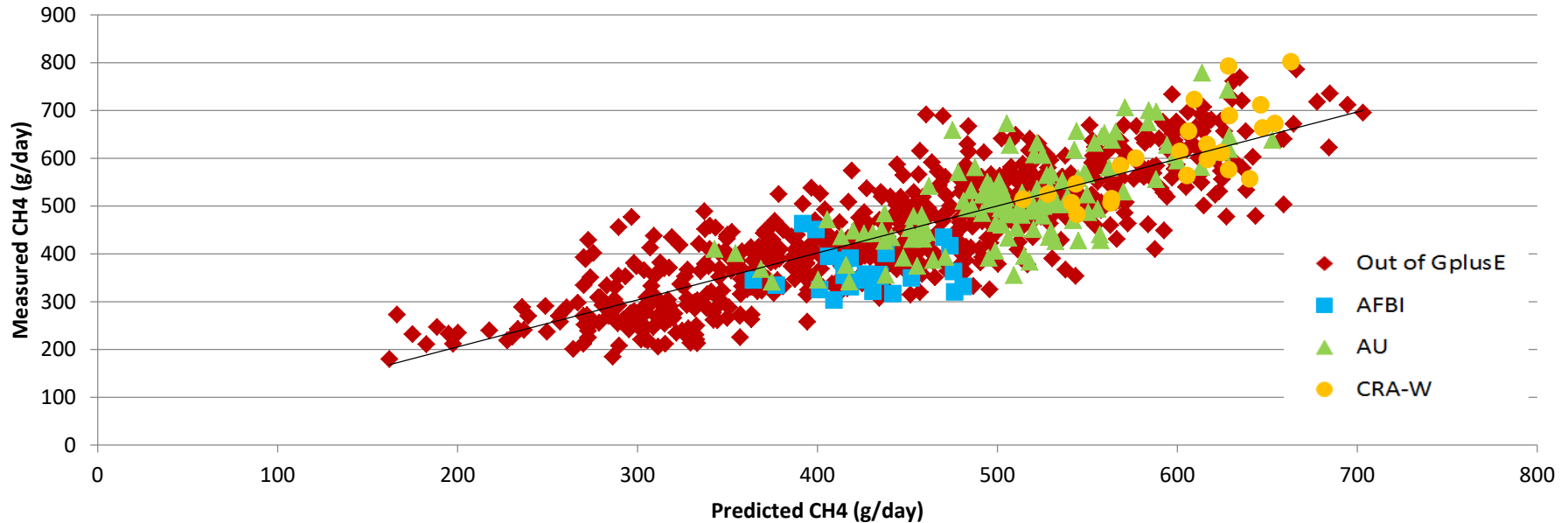
Constituent	COW	N	Mean	Min	Max	SD
AU	19	132	512	341	779	90
AFBI	12	24	365	304	463	44
CRA-W	4	24	618	483	802	93



# Direct prediction of phenotypes

## ◦ Methane (CH<sub>4</sub>)

Constituent	N	Mean	SD	R <sup>2</sup> c	R <sup>2</sup> cv	SEC	SECV
CH <sub>4</sub>	863	459	123	0.71	0.67	66	71



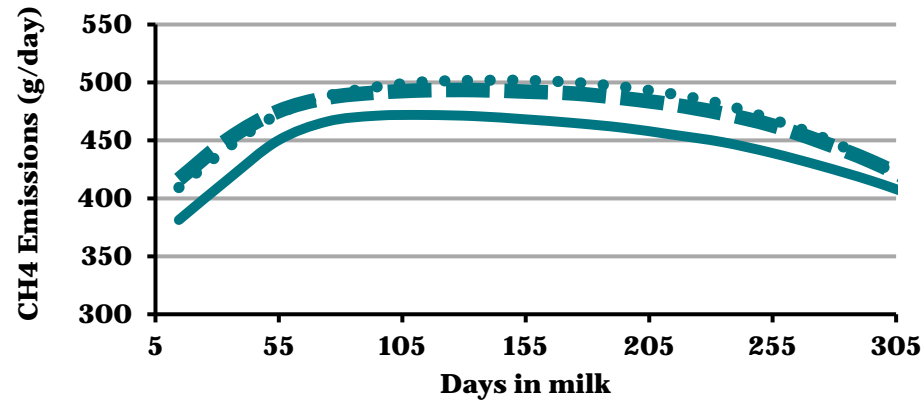
# Example of large scale application

## Methane

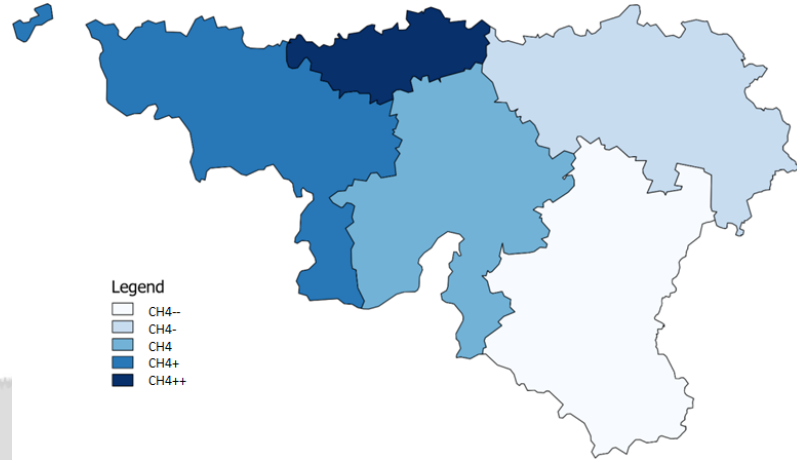


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M. L. Vanrobays

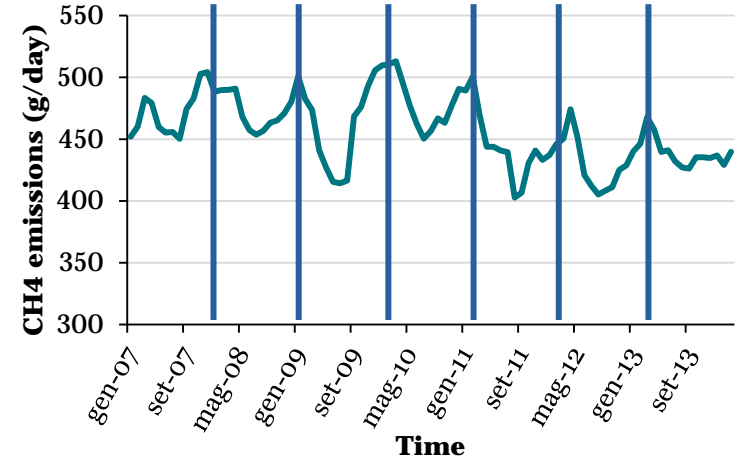


— Lactation 1    - - - Lactation 2    . . . Lactation 3



Legend

- CH4-
- CH4-
- CH4
- CH4+
- CH4++



Time

# Conclusion

- Still work to do but real potential use of MIR to predict phenotypes of interest
- Some firsts examples... but many more! (Milk IsoC, LDH, Energy and Protein efficiency, confirmation of pregnancy, loss of weight, etc.) + Interactions
- Calibration (“exact” value) – Classification (+ or -) – Threshold (limit)
- Interest - in the framework of GplusE (link with genotype)
  - for milk recording and farmers (easy alarms)
  - for global studies (“historic” of milk spectra)



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