Conffidence

Cluster 2 -Veterinary Pharmaceuticals

Coccidiostats (WP2a)

Antibiotics (WP2b)

27th January 2010




SEVENTH FRAMEWORK PROGRAMME

www.conffidence.eu

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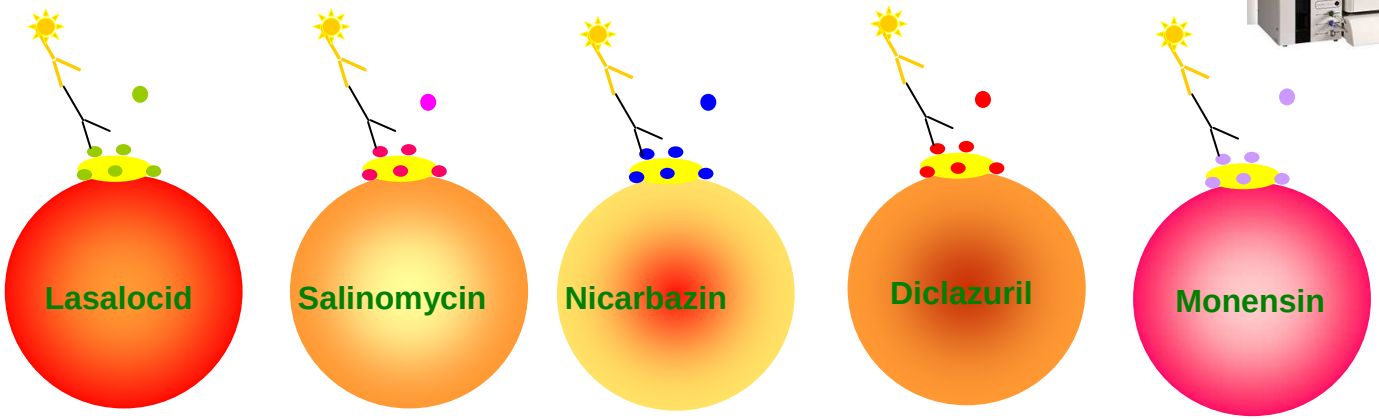
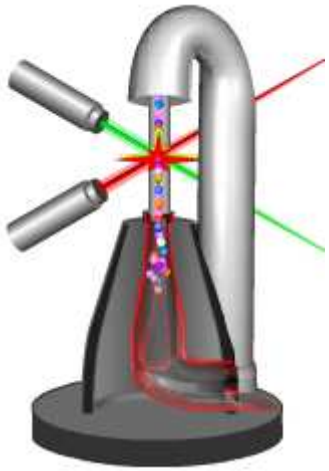
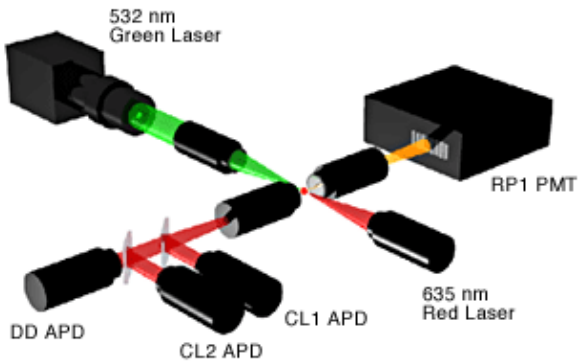
WP2a objectives

- Validated flow cytometry based multiplex immunoassay for residues of lasalocid A, monensin, salinomycin, narasin and nicarbazin in eggs and their
- Cross- contamination in non-targeted feed (laying hens feed)
- Simplified sample preparation protocols for eggs and feed
- Carry-over study of lasalocid from laying hens feed to eggs aiming at contribution to a predictive hazard behaviour model.

Flow cytometry based immunoassay







APD: Avalanche photodiode detector

DD: Doublet discriminator channel, discriminates single beads from aggregated beads

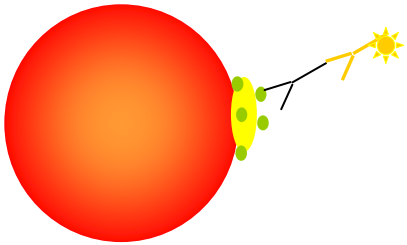
CL1: Classify channel, allows multiplexing, detects dye inside beads

CL2: Classify channel, allows multiplexing, detects dye inside beads

RP1: Reporter channel, quantitates assay in this channel

Results and conclusions

- New antibodies
  - Lasalocid — IC50 = 1 ng/ml
  - Monensin — To low max. response
  - Narasin — Ready to be tested
- 4 assays (nicarbazin, diclazuril, salinomycin and lasalocid) are ready to be tested with sample materials.
- Diclazuril and nicarbazin antibodies showed no cross-reaction with 10 other coccidiostats.
- Salinomycin antibody only showed cross-reaction with narasin (2%).
- Lasalocid antibody will be tested for cross-reaction.



RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

CONFIDENCE

Dipstick assays for antibiotics

Fera, UK *(WP leader)*

Unisensor, Belgium *(technology)*

CSIC, Spain *(technology)*

Nestlé, Switzerland *(food industry)*

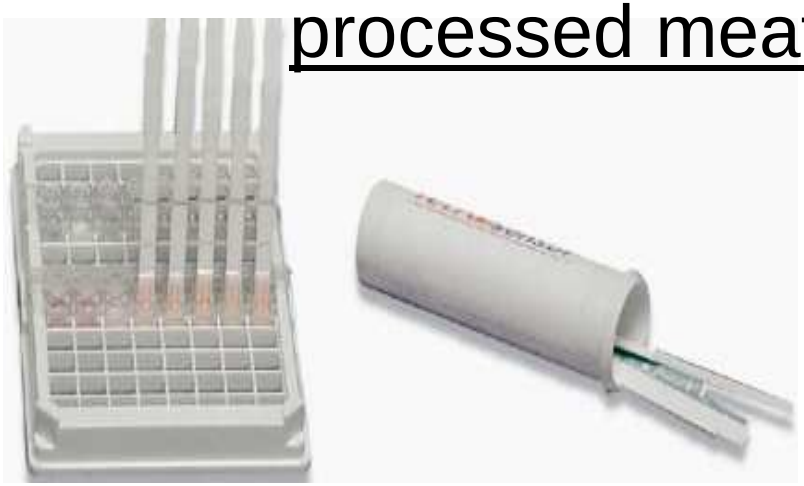
CER, Belgium *(antibody reagents)*

Nutreco, The Netherlands *(animal feed industry)*

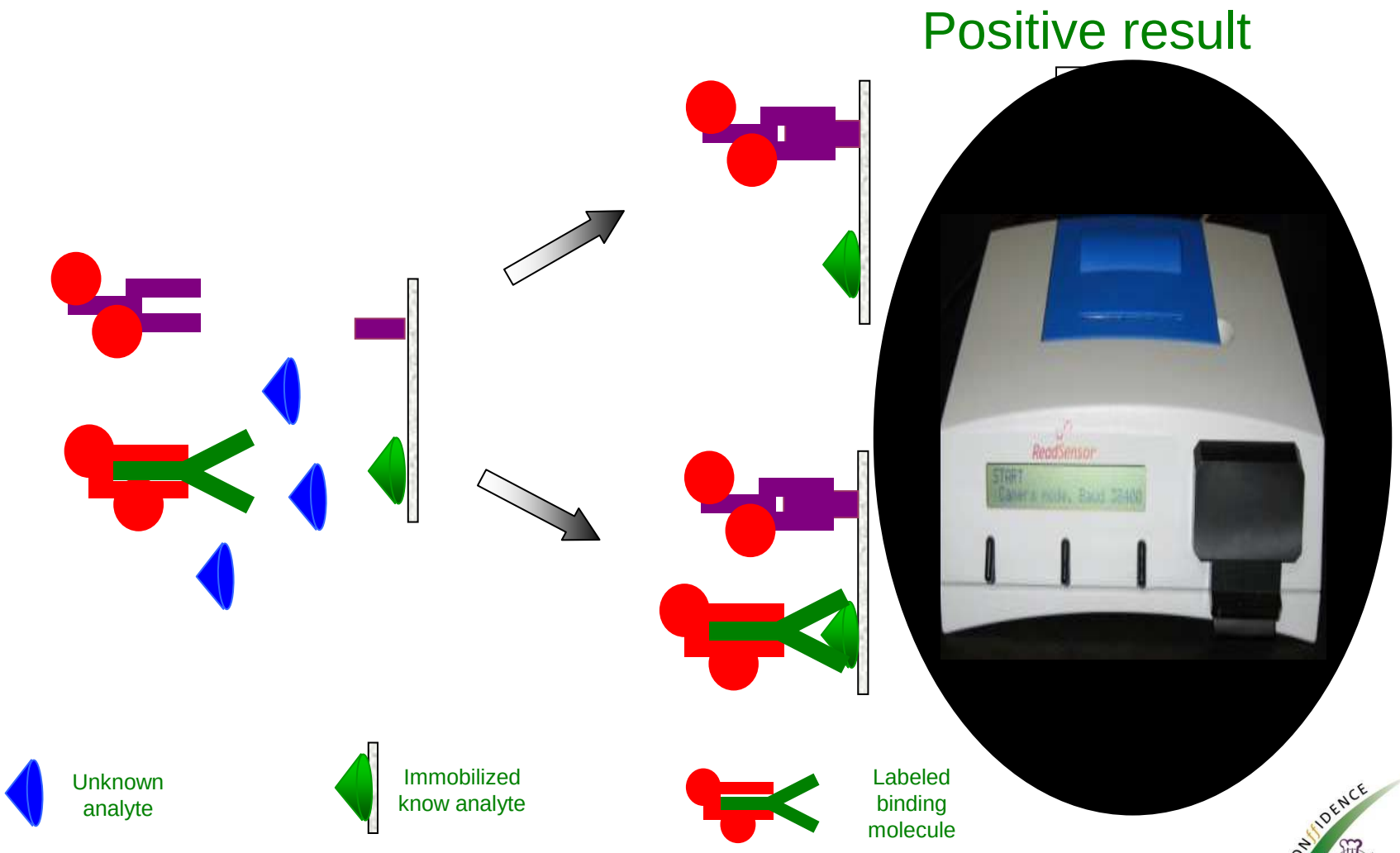
Joint Research Council (EC), Belgium *(validation)*

WP2b antibiotics - Objective

"Development, validation and impact demonstration of single-component and multiplex dipsticks to detect **malachite green, tetracyclines, tylosin, chloramphenicol, quinolone and sulfonamide** antibiotics in a range of matrices including fish, feeds, urine, processed meat and honey"



Dipstick technology mechanism of action



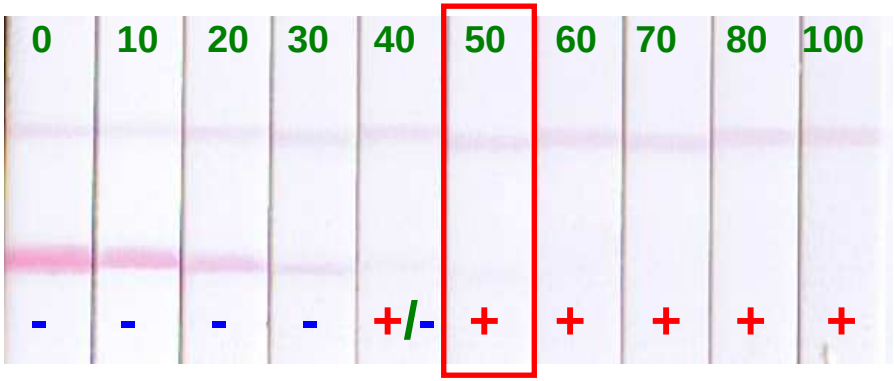
Tetrasensor® for multi-tetracyclines

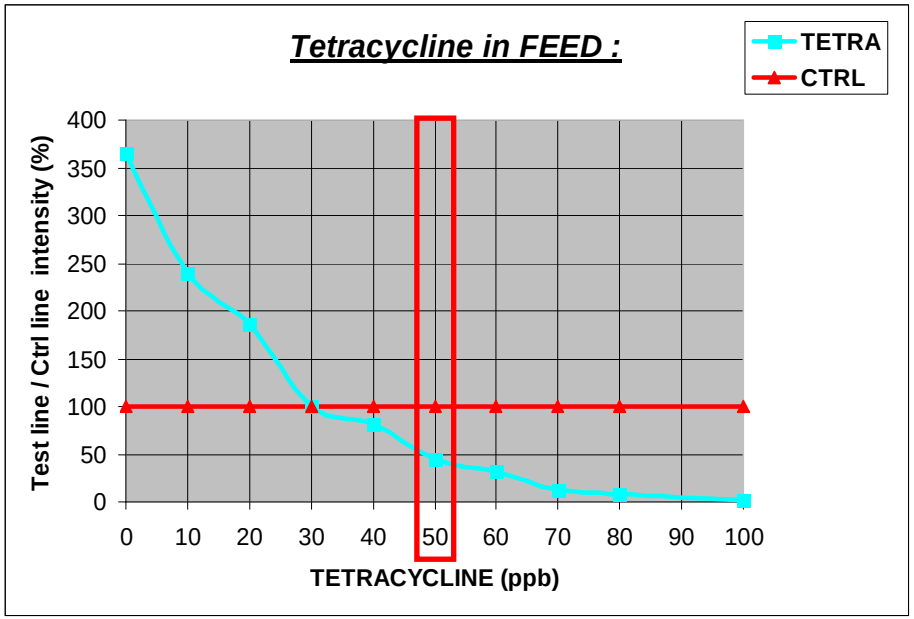
http://www.tetrasensor.com/

Receptor (TetR) based dipstick for;

oxytetracycline, tetracycline, chlortetracycline, doxcycline, minocycline & others

In muscle, kidney, feed, honey, urine, milk





Simple assay protocol

Sample **-** dilution (**10x)** in buffer

Homogenise (2 min)

Centrifugation (2 min)

200 µl of sample + reagents

Test : **10 min** at **RT**

Tetrasensor® limits of detection

Matrix	Limit of detection ($\mu g\ kg^{-1}$)	*Target Conc.* ($\mu g\ kg^{-1}$)	Time (min)	
			Preparation	Analysis
Muscle	20 -100	50	**5**	**10**
Honey	10	20	**1**	**30**
Urine[2]	50	100	**1**	**10**
Feed[2]	50	100	**5**	**10**
Milk	20 -100	50	**1**	**10**

[1] *LOD for least sensitive compound tetracycline quoted (TC<OTC/CTC<DOX)*

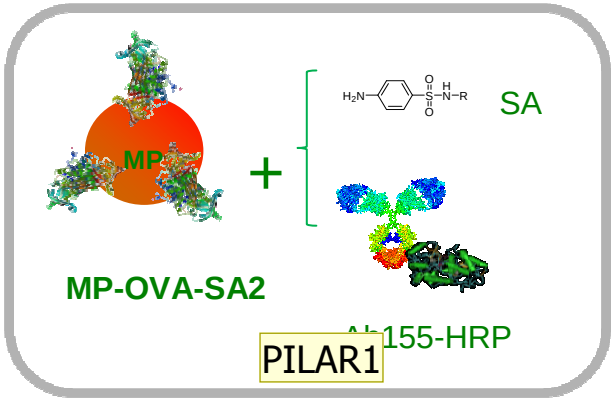
[2] *Methods currently under evaluation*

Sulfasensor® for sulfonamides

Prototype assay

- Competitive antibody based **dipstick** assay
- Detects more than **10** sulfonamides in honey including;
  sulfamethazine, sulfathiazole,sulfamerazine, sulfachlorpyridazine,sulfamonomethoxine, sulfamethoxypyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaquinoxaline
  - At 25 µg kg^{-1} or less
- **5 min** of sample processing (hydrolysis)
  (trichloroacetic acid, 5 min at *ca.*100^{o} C)
- **20 min** of analysis

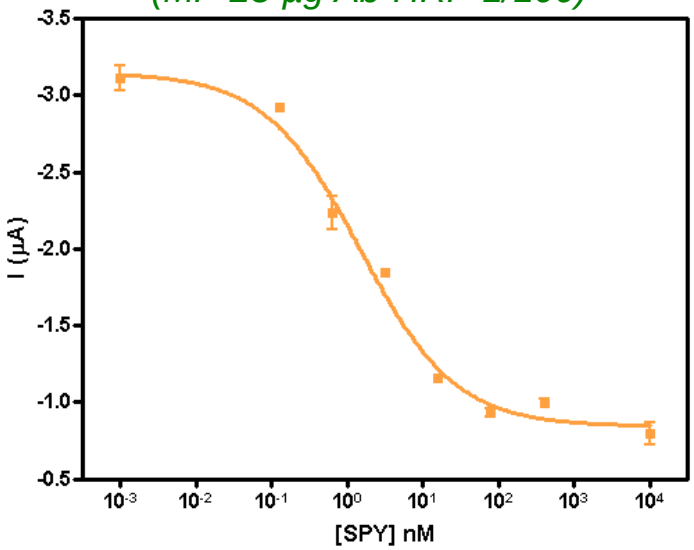
Electrochemical Magneto Immunosensor (ECMIS) sulfonamides in honey



Calibration Curve

Hydrolysed Honey (1/10 PB, 5min 100ºC)

(MP 25 µg Ab-HRP 1/100)



Assay Characteristics

- Easy sample processing :
  - *hydrolysis & conditioning* (15 min)
- Total analysis time: 30 samples in 3h 15 min.
- Repeatability: CV 8.9%
- Selectivity:
  - *Up to11 sulfonamides are detected in honey at <25 µg kg^{-1}*
- Detection Capability (CCβ) :18.5 µg Kg^{-1} *(using SPY as reference)*

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PILAR1 Insertar gráfica
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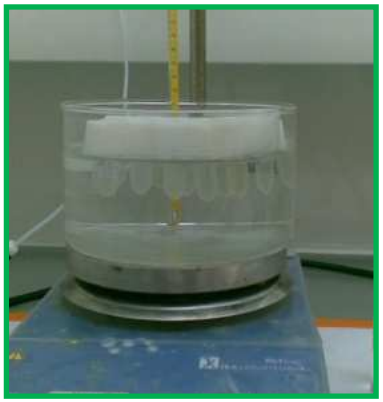
ECMIS protocol for sulfonamides in honey

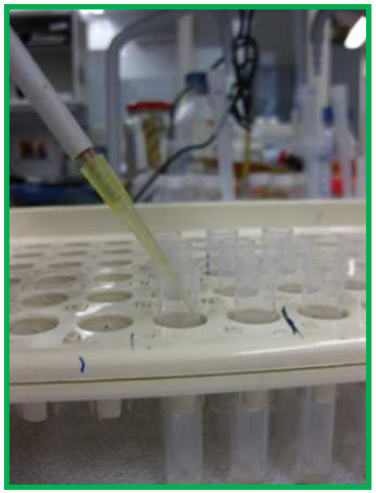
1

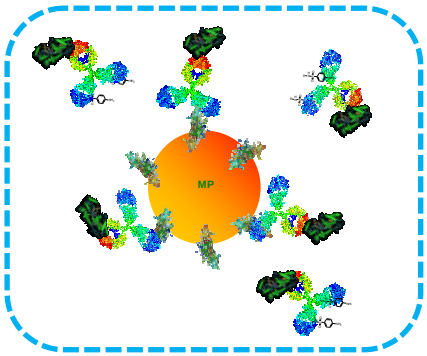


Hydrolyis:
2 N HCl 5 min/ 100 ºC
Conditioning:
Neutralization & Buffering

30 samples/15 min

2



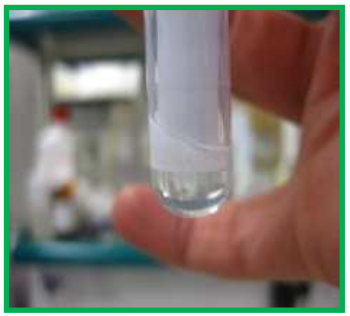


Immunochemical Assay

Ab-HRP
+
SA2-OVA-MP

30 samples/45 min

3



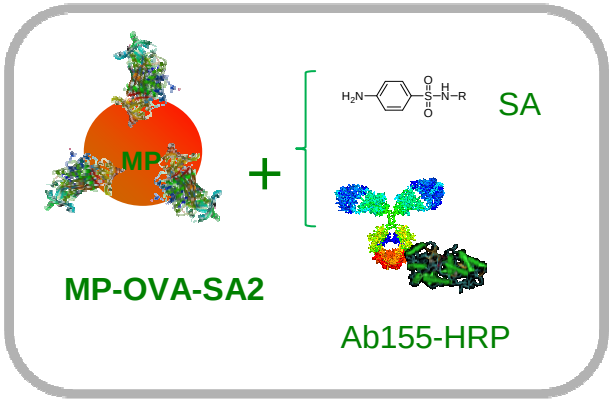
Capture the MP with m-GEC electrode

4

Amperometric Mesures
& Data Analysis
4 min / sample

TOTAL ANALYSIS TIME:
3 h 15 min/ 30 samples

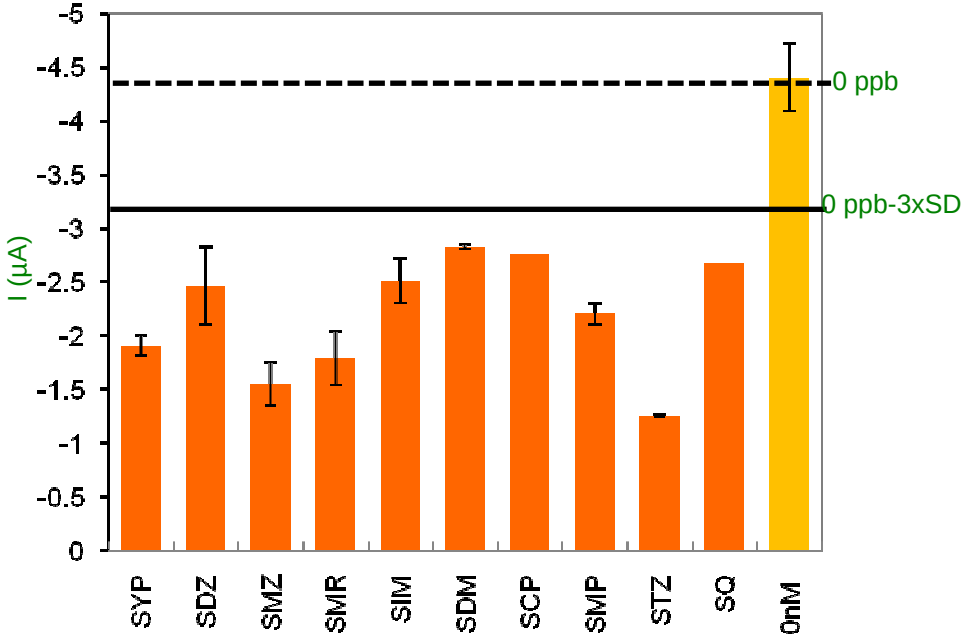
ECMIS specificity in honey



10 different SAs were detected below 25 ppb, with an amperometric signal below 3 times the SD of the control zero.

10 Sulfas at 25 ppb

Blank spiked Honey 2N HCl (1/10 PB)



Future plans and dissemination activities

- Dipstick device for MG/LMG in fish
- Multi-sensor (dipstick array) for antibiotics in honey (sulfas, FQs, CAP, tylosin)
- Assay validation
- Predictive hazard modelling study (on farms/ retail samples)

- Presentations at Ghent symposium on hormone and veterinary residue analysis (June 2010)
- Training workshops