

# VMO2160: Confidence

Work package 2b - Antibiotics

## "Rapid Testing for Antibiotics In Honey - A Real Field Test"

National Bee Unit - Technical Training Conference - 2012  
29/03/12 - at Fera, Sand Hutton, UK



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# Outline

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- Background on the *Confidence* project
- Background on conventional analysis of honey
- Overview of the lab format of the new screening assay
- Overview of the proposed field test format
- Summary & Outlook
- Acknowledgements
- Demonstration of the test kit
- **Wanted!**

**Volunteers to evaluate field test kits- summer 2012**



# CONFIDENCE project

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- **CONTaminants in Food and Feed : Inexpensive DETection for Control of Exposure...**
- **Collaborative Project** : FP7 - European Commission
- **Duration**: 4 years (May 2008 – April 2012) > extension Dec 2012
- **Partners**: 16 partners from 10 countries (universities, SME, research institutes,...)
- **Budget**: 7.5 Mio €
- **Coordinator**: RIKILT - Institute of Food Safety (NL)
- **Objective**: Development of innovative, reliable, simple, fast and multiple screening tests for chemical contaminants and residues in food and feed



# WP2b overall objective

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“Development, validation and **impact demonstration** of single-component and multiplex dipsticks to detect

- malachite green
- tetracyclines
- **tylosin,**
- **chloramphenicol,**
- **(fluoro)quinolones**
- **sulfonamides**

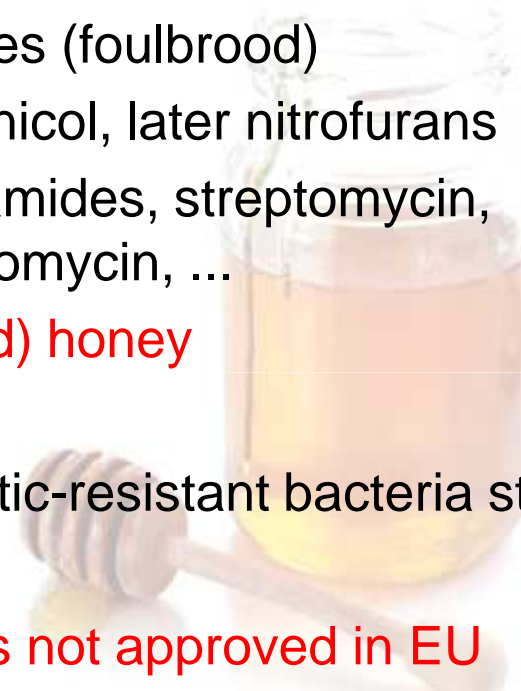
in a range of matrices including fish, feeds, urine and processed meat and **honey**”



# Antibiotics in Honey



- Use of antibiotics by some beekeepers to treat or prevent bacterial infestations of hives (foulbrood)
- 2002/03 alerts relating to chloramphenicol, later nitrofurans
- Known usage of tetracyclines, sulfonamides, streptomycin, tylosin, quinolones, lincomycin, erythromycin, ...
- **Multiple antibiotics present in (blended) honey**
- Concerns about emergence of antibiotic-resistant bacteria strains
- **The use of antibiotics in beekeeping is not approved in EU**
- Other countries have set Residue Limits
- Testing required in all cases!



# Conventional analysis of honey

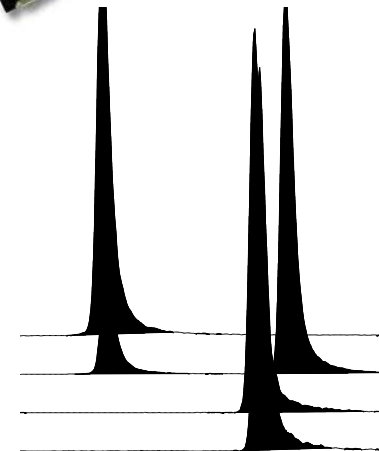
## Day 1

1. Dissolve honey in water/solvent
2. Clean-up (solid phase extraction)
3. Evaporation step of the organic solvent
4. Reconstitution of the extract
5. Analysis by LC-MS/MS

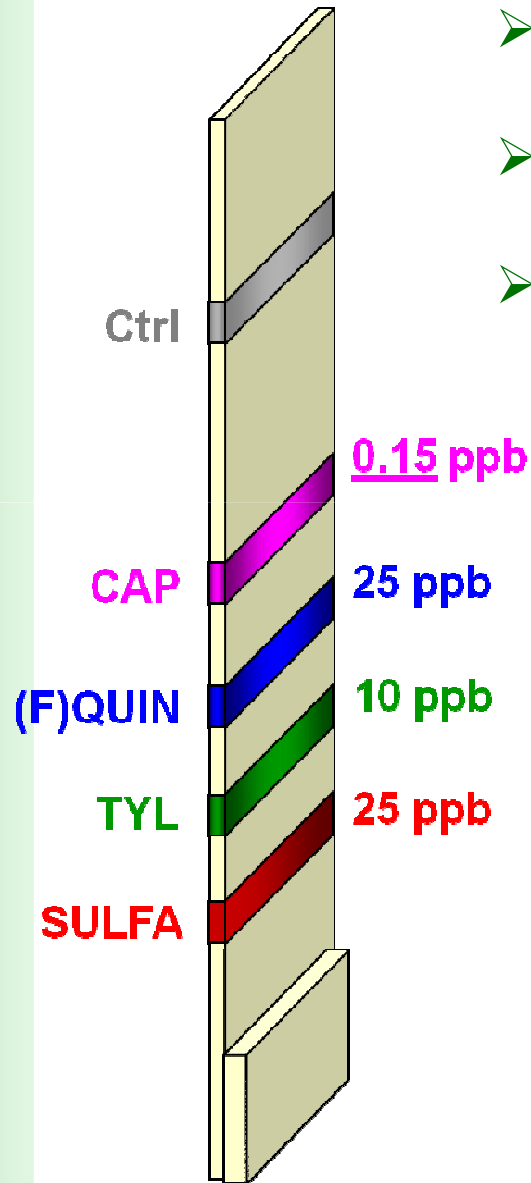


## Day 2

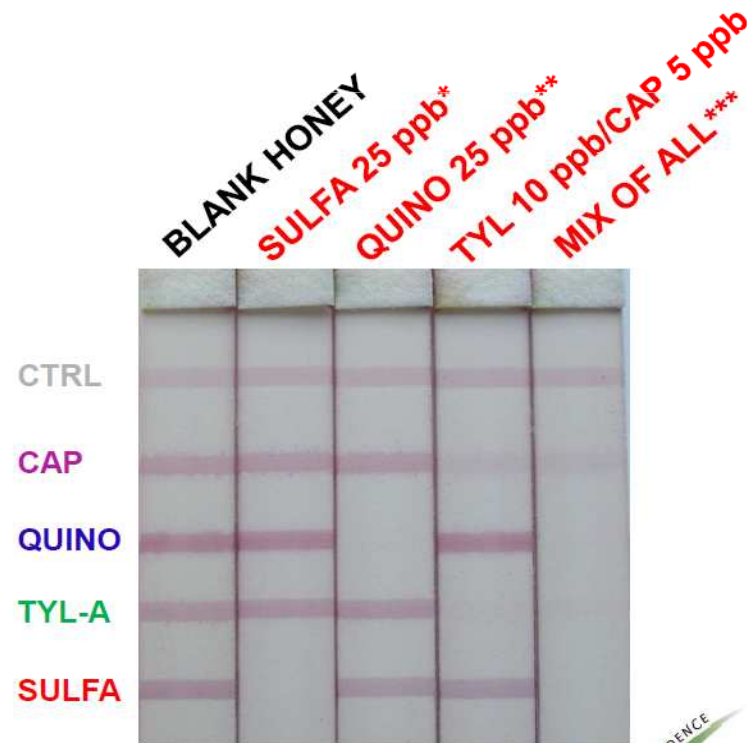
6. Process the data



# Multiplex assay concept



- Competitive inhibition format (Lateral flow device);
- Incorporating 4 test lines and 1 control line;
- Exploiting matched pairs of antibodies and analyte-protein (OVA) competitors;



# Lab method overview - multisensor


- Two aliquots (A and B) are required.
- (A) is dissolved using acid hydrolysis
- (B) is dissolved in water.
- Liquid/liquid partitioning with ethyl-acetate,
- Evaporated to dryness under nitrogen
- (A) and (B) are combined – applied to test kit for 10 minutes at 40°C





## Field-test

# ~~Lab~~ method overview - multisensor

- Two aliquots (A and B) are required.
- (A) is dissolved using acid hydrolysis
- (B) is dissolved in water.
- 
- (A) and (B) are combined – applied to test kit for ~~10 minutes at 40°C~~ **20 min at RT**



# Multisensor 'Field Test' kit content:

## General equipment

Floation device, bulb pipette, spatula, dipstick well rack, thermos flask, 2 x mugs, gloves and safety glasses

## Containers for reagents

Water tube: **Pink** lid.

Concentrated buffer tube:            lid.

Neutralising buffer: **Red** lid. (warm in boiling water 10min).

## Containers for samples

A: Acid hydrolysis tube: **Blue** lid

    Lid for weighing honey (acid hydrolysis): **Blue** lid

B: Non acid hydrolysis tube: **Purple** lid

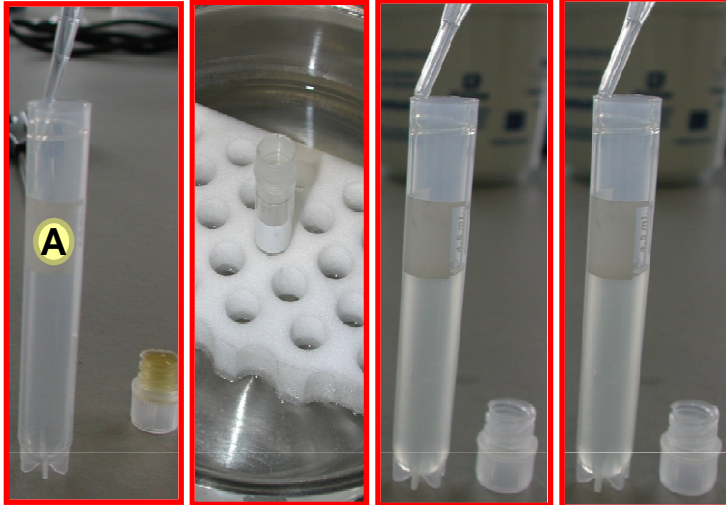
    Lid for weighing honey (non-acid hydrolysis): **Purple** lid.

C: Mixing tube: **Green** lid



# Field-test : method schematic

## 1. DILUTION / HYDROLYSIS

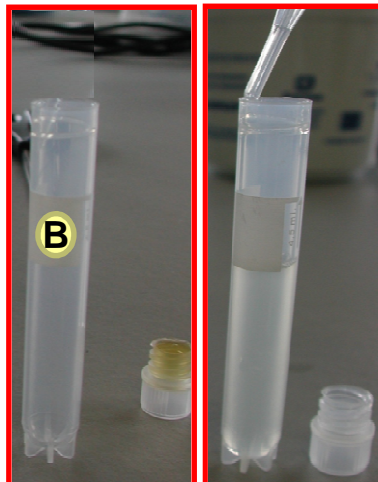


0,65 gr  
HONEY

300  $\mu$ l Acid  
Hydrolysis  
(5 min 95°C)

300  $\mu$ l Base  
Neutralization

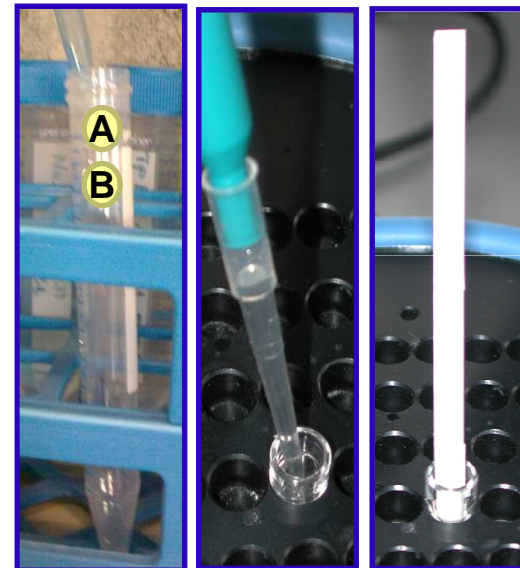
2,4 ml buffer  
Dissolution



0,65 gr  
HONEY

3 ml buffer  
Dissolution

## 3. DIPSTICK



Mix A & B  
200 $\mu$ l/200 $\mu$ l

5 min  
Incubation  
at 25°C (RT)

15 min  
Dipstick  
At 25°C (RT)

<30 min  
TOTAL

All material  
provided in  
the kit !



# Test procedure

Take **blue** (A) and **purple** (B) lid and fill cap with honey using a spatula.

Transfer concentrated buffer (      lid) into the water (**pink** lid). Mix by inversion.

Remove **blue** lid from tube A (containing acid) and screw **blue** lid containing honey on. Mix by inversion.

Fill tube **B** to 3 ml line with diluted buffer solution.

Screw **purple** lid onto tube B and mix by inversion.

Place both tubes **A** and **B** into hot water ( $>90^{\circ}\text{C}$ ) for 5mins.  
**Important:** Do not to leave longer than 5mins.



## Test procedure continued.

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Remove both tubes from water and allow to cool for 5-10min.

Neutralize tube **A** with 6 drops of neutralising buffer (**red** lid).  
Mix by inversion

Fill (**A**) up to 4.5ml line with diluted buffer solution.

Fill (**B**) up to 4.5ml line with diluted buffer solution.

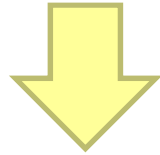
**Option 1:** Fill up to 1ml line on tube **C** with liquid from tube **A** then fill up to 2ml line with liquid from tube **B** (1:1 ratio of **A** and **B**)

**Option 2:** Combine total contents of **A** and **B** into tube **C**.



# Test procedure continued.

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Transfer 200 $\mu$ l of mixture to the micro-well and leave for 5 min.



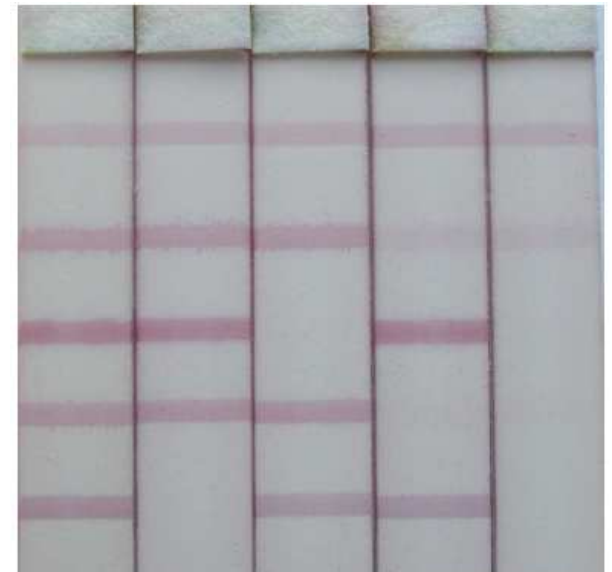
Add dipstick to the well and leave for 20 min.



Remove from test well and scrape off absorbent pad.



Visual assessment



# Comparison of detection capability

antimicrobial	LC-MS/MS [µg/kg]	Multi-lab [µg/kg]	Multi-field [µg/kg]
Sulfathiazole	2.5	25*	50
Tylosin	2	10	50
Ciprofloxacin	10	25#	50
Chloramphenicol	0.15	5	100
<b>Time required</b>	<b>3x2 Days</b>	<b>more than 4h</b>	<b>less than 1h</b>

\*Applicable to 11 other sulfonamides

#Applicable to 5 other fluoroquinolones and 3 at 100 µg/kg



# Summary & Outlook

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- Real field test – multiplex dipstick assay (LFD), available shortly for trial
- Rapid detection of antimicrobials
  - Prevention of mixing honeys containing residues with blanks
- **Wanted!** Minimum of **ten** bee-inspectors to take test-kit into the field in **summer 2012**
  - a variety of locations and floral types
  - to test in total: 10 x 6 hives by NBU
- Parallel experiments in other countries





# Thanks to...

- Multiplex dipstick development :
  - **UNISENSOR S.A.** (Belgium)
  - **CER** (Belgium)
  - **CSIC** (Spain)
- Matrix preparation & **lab validation** :
  - **FERA** (United Kingdom)
  - **NESTLE NRC** (Switzerland)
- Project coordination :
  - **RIKILT** (The Netherlands)
- Funding :
  - **CONFIDENCE** (European Commission FP7 Grant agreement n°211326)



# Thank you for your attention!



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