

Confidence – Workshop:

Work package 2b - Antibiotics

Detection, analysis and control of veterinary pharmaceuticals in food and feed

‘Validation of **bee4sensor** for Honey’

EuroResidue VII – 16 May 2012

Hotel Zuiderduin, Egmond aan Zee, The Netherlands



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Outline

- Overview of format of the new screening assay
- Single laboratory validation data
- Inter-laboratory validation (ILV) data
- Overview of a new 'field-test format'
- Summary & Outlook
- Acknowledgements

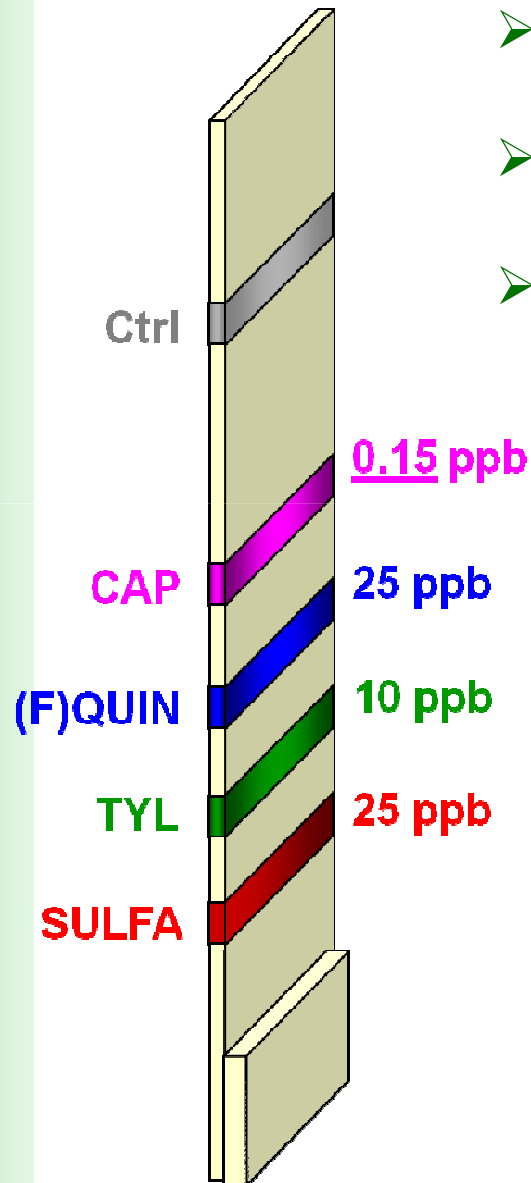


Outline

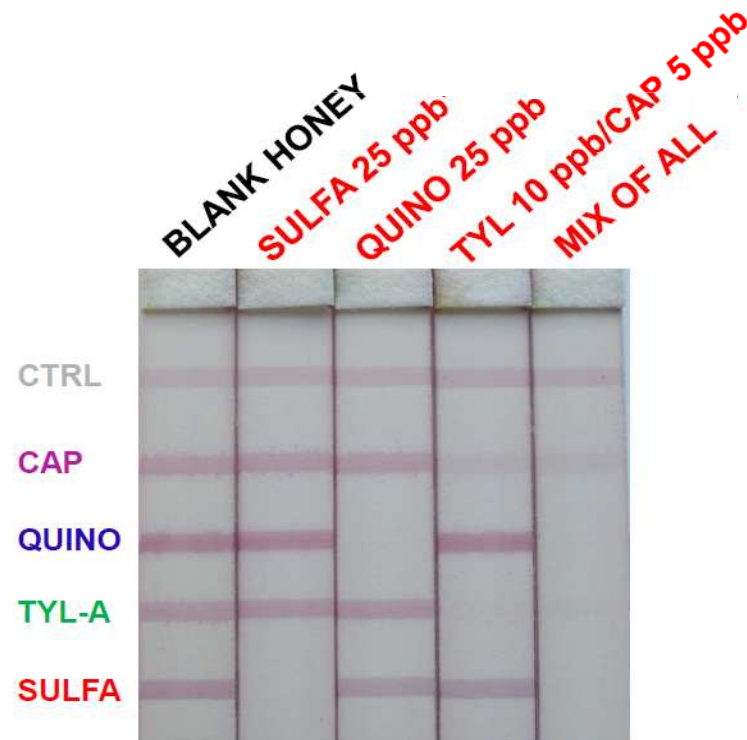
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Multiplex assay concept



- 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 – bee4sensor

- 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



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Summary of single lab validation (CC β)

Lab method

	Sulfonamides	Macrolides	Fluoroquinolones	Amphenicols
	Sulfathiazole	Tylosin	Ciprofloxacin	Chloramphenicol
CC β [μ g/kg]	25	10	25	5

N = 60 (3 different honeys, n=10 each analysed over 6 days)



Summary of single lab validation – LoDs

Lab method

Sulfonamides	LoD [µg/kg]
Sulfathiazole	25
Sulfadiazine	25
Sulfapyridine	25
Sulfamerazine	25
Sulfamethazine	25
Sulfamethoxypyridazine	25
Sulfachlorpyridazine	25
Sulfamonomethoxine	25
Sulfadimethoxine	25
Sulfamoxole	25
Sulfaquinoxaline	25

Fluoroquinolones	LoD [µg/kg]
Ciprofloxacin	25
Danofloxacin	25
Enrofloxacin	10
Nalidixic acid	25
Norfloxacin	10
Oxolinic acid	25
Flumequine	100
Marbofloxacin	100
Difloxacin	100

Others	LoD [µg/kg]
Tylosin	10
Chloramphenicol	5



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Small scale inter-lab validation (ILV) study

Lab method

- Incurred and spiked test materials prepared
- Seven participants (UK, 2 x BE, CH, NL, 2 xFR)
- Laboratories from industry and official control organisations
- Blind samples
- Visual and instrument (Readsensor®) measurements



Summary of ILV

Lab method

➤ n = 42, total number of replicates per STC

analyte	match of expected and measured outcome				
	STC	visual [%]			
	[µg/kg]	Blank	0.5x STC	1xSTC	
sulfathiazole	50	100			
tylosin	20		100		
ciprofloxacin	50				
chloramphenicol	10		97		

STC = Screening Target Concentration



Summary of ILV

Lab method

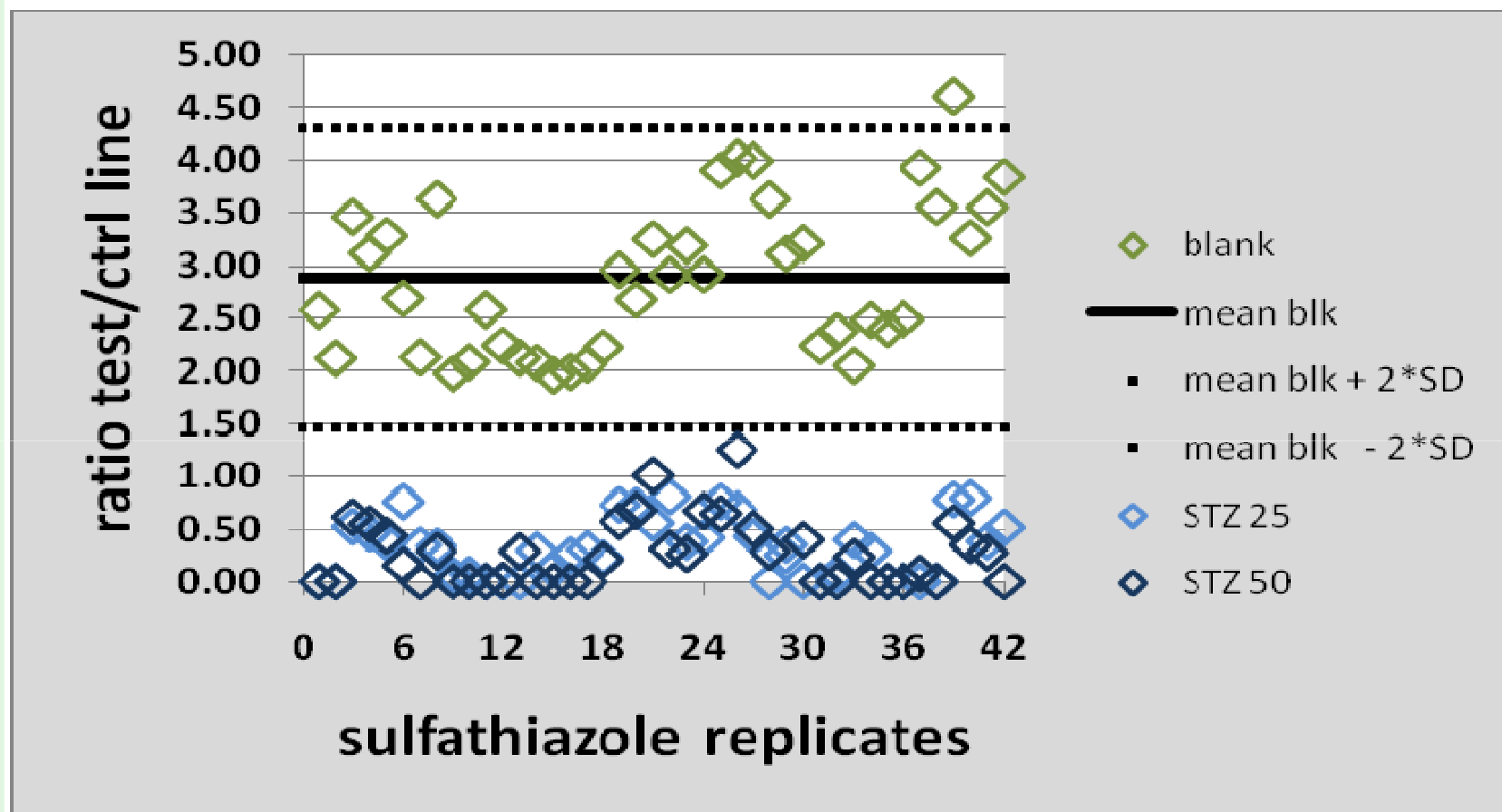
➤ n = 42, total number of replicates per STC

analyte	match of expected and measured outcome						
	STC	visual [%]			Readsensor® [%]		
	[µg/kg]	Blank	0.5x STC	1xSTC	Blank	0.5x STC	1xSTC
sulfathiazole	50	100	100	100	100	100	97
tylosin	20					95	
ciprofloxacin	50		97			100	100
chloramphenicol	10					93	

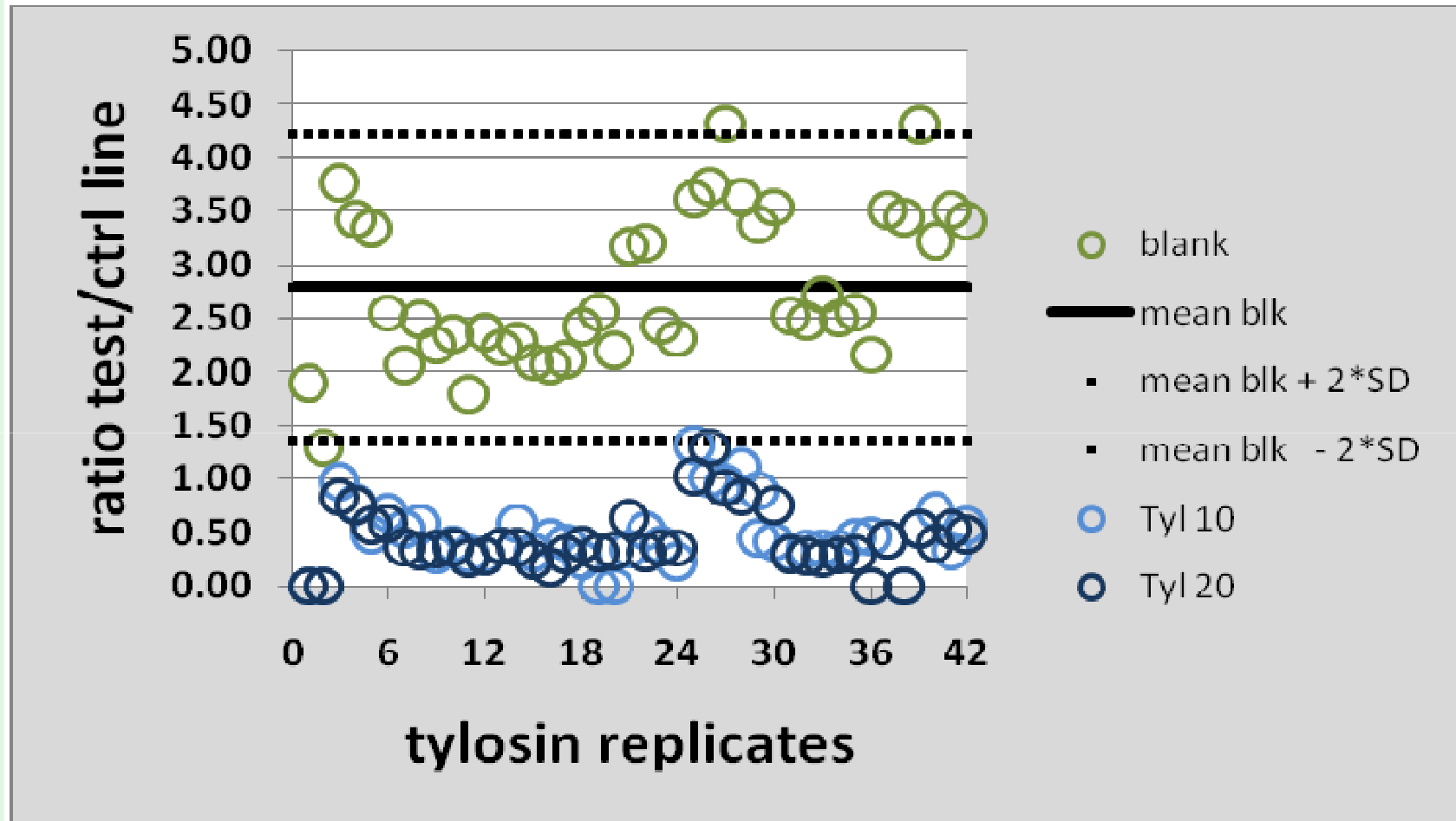
STC = Screening Target Concentration



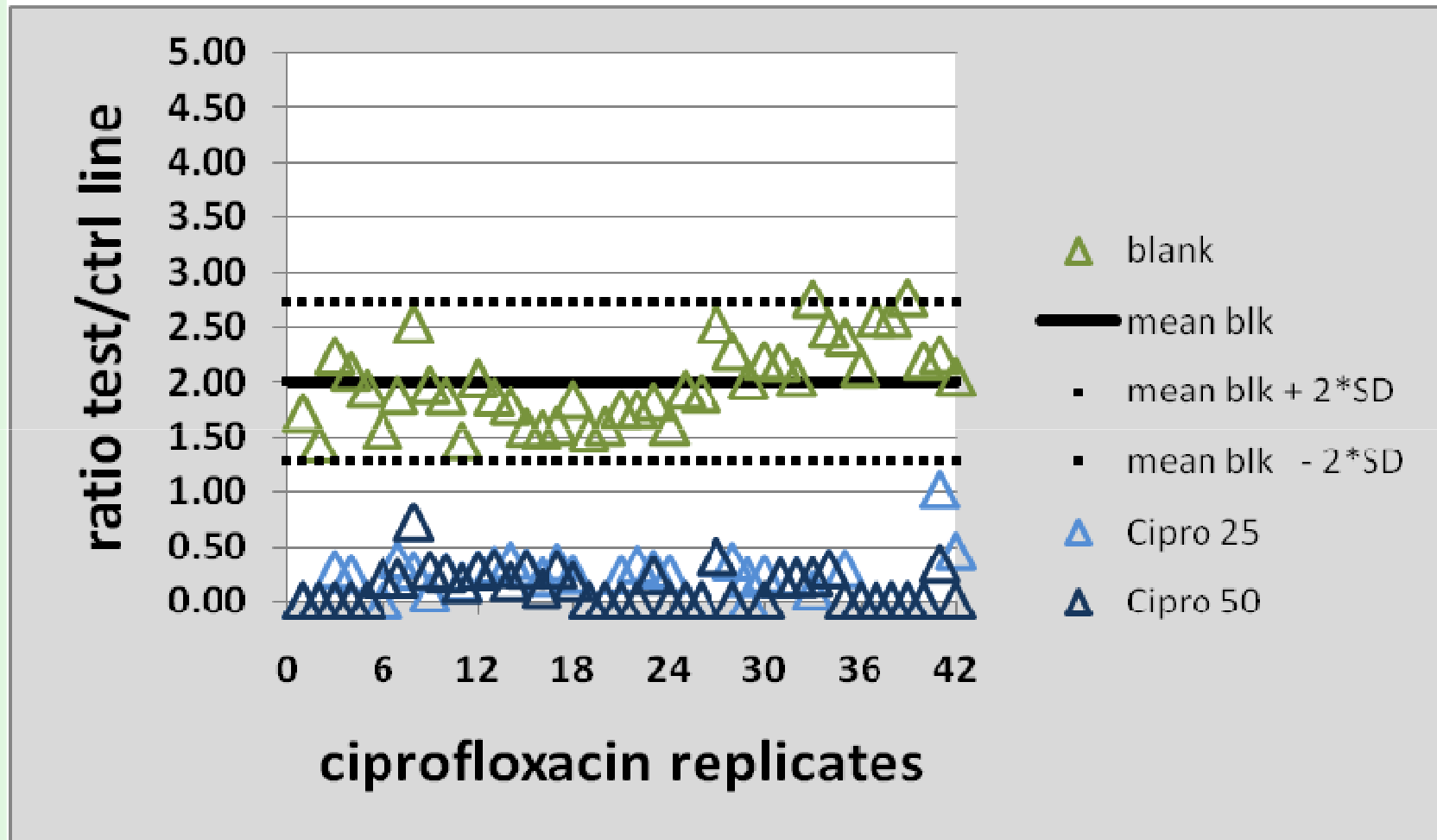
ILV- sulfathiazole by Readsensor®



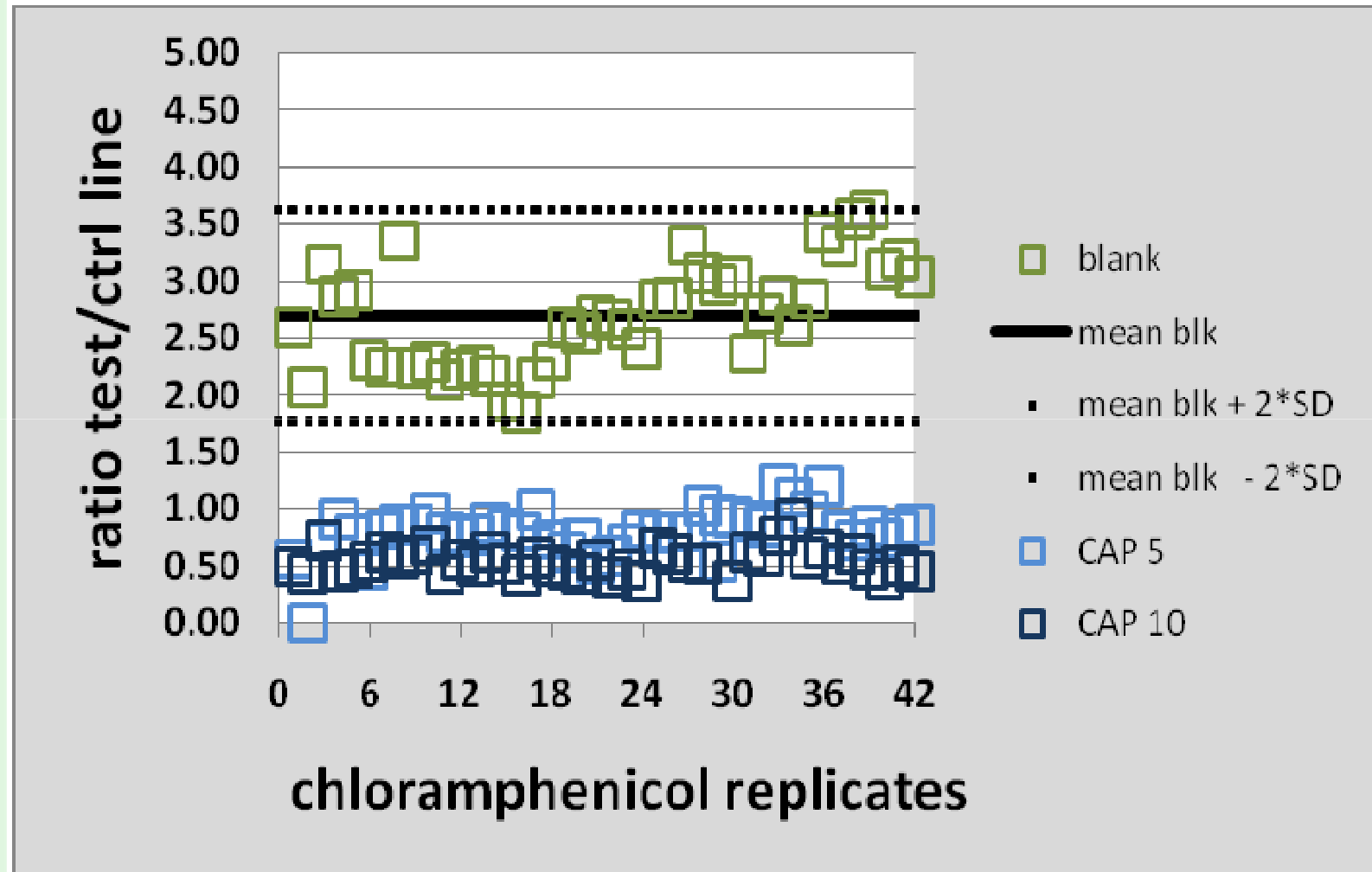
ILV- tylosin by Readsensor®



ILV- ciprofloxacin by Readsensor®



ILV- chloramphenicol by Readsensor®




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Field-test

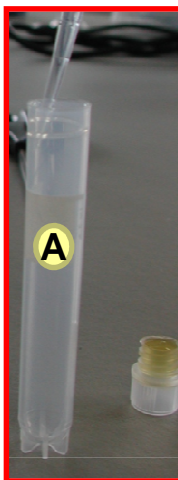
~~Lab~~ method overview – bee4sensor

- 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**



Field-test : method schematic

1. HYDROLYSIS / DILUTION



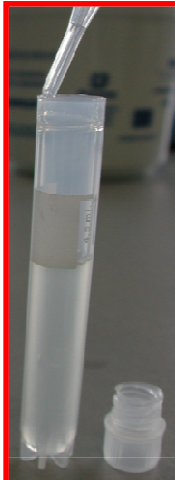
0,65 gr
HONEY



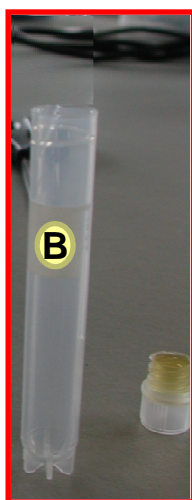
300 µl Acid
Hydrolysis
(5 min 95°C)



300 µl Base
Neutralization



Final extract
volume, 3.5 ml



0,65 gr
HONEY



2ml dissolution
buffer
(5 min 95°C)



Final extract
volume, 3.5 ml – made up with buffer

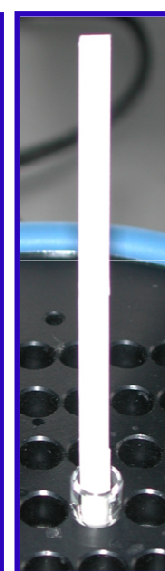
2. DIPSTICK



Mix A & B
1:1 ratio



5 min
Incubation
at RT



20 min
Dipstick
at RT

**<30 min
TOTAL**

**All material
provided in
the kit !**



Comparison of detection capability

antimicrobial	Lab method [µg/kg]	Field method [µg/kg]
Sulfathiazole	25*	50
Tylosin	10	25
Ciprofloxacin	25#	25
Chloramphenicol	5	100
Time required	more than 4h	less than 1h

*Applicable to 11 other sulfonamides

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



Summary & Outlook

- Rapid detection of antimicrobials in honey
- Real field-test – multiplex dipstick assay (LFD), available shortly for trial
- Minimum of ten bee-inspectors will evaluate the test-kit in the field in **summer 2012**
 - a variety of locations and floral types
- 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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