

## DEVELOPMENT OF A NEW HIGHLY SENSITIVE ELISA FOR THE RAPID SCREENING OF TETRACYCLINE IN HONEY

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### INTRODUCTION

Tetracycline is a broad-spectrum antibiotic used in apiculture for treatment of bacterial diseases, such as foulbrood. The use of antimicrobials in beekeeping is strictly regulated or banned. The main purposes for monitoring bee products are for consumer health protection, international commercial competition, and better product quality.

This study reports the development of a new highly sensitive, rapid one step, competitive Enzyme-Linked Immunosorbent Assay (ELISA) for the screening of tetracycline residues in honey.

### METHODOLOGY

With this competitive ELISA the assay time is 1 hour. The analyte present in the standard or sample competes with horseradish peroxidase labelled conjugate for a limited number of antibody sites (antibody solution). The antibody then binds to a second antibody immobilised and stabilised on a 96-well microtitre plate surface. Absorbances were read at 450 nm. A standard series, spiking material and ready to use conjugate are included.

Shelf life stability was assessed by accelerated stress, each component was stressed at 37°C for 7 and 14 days related to the same material stored at +2-+8°C.

Sample preparation: 1g of honey is dissolved in 9 ml of diluted diluent/wash buffer by rolling for 10 min. The supernatant is diluted 1:1 with sample diluent and then mixed before analysis. Sample volume per well 50µL.

### RESULTS

#### Sensitivity

##### Limit of Detection (LOD)

Analyte	Matrix	Tetracycline equivalence (ppb)
Tetracycline	Honey (dilution factor 20)	2

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##### IC<sub>50</sub>

Analyte	Calibration Range (ppb)	IC <sub>50</sub> (ppb)
Tetracycline	0 - 2.04	0.15

6640-QC

#### Specificity/Cross-reactivity (CR)

Compound	CR (%)
Tetracycline	100
4-epitetracycline	87
Rolitetetracycline	67
4-epioxytetracycline	52
Oxytetracycline	52
Chlortetracycline	51
Demeclocycline	41
Doxycycline	23
4-epichlortetracycline	20
Methacycline	11
Minocycline	<1
Norflaxacin	<1
Ceftiofur	<1
Florfenicol	<1
Streptomycin	<1
Tylosin	<1

TCS101.17.IFU.16091.fsm

#### Recovery

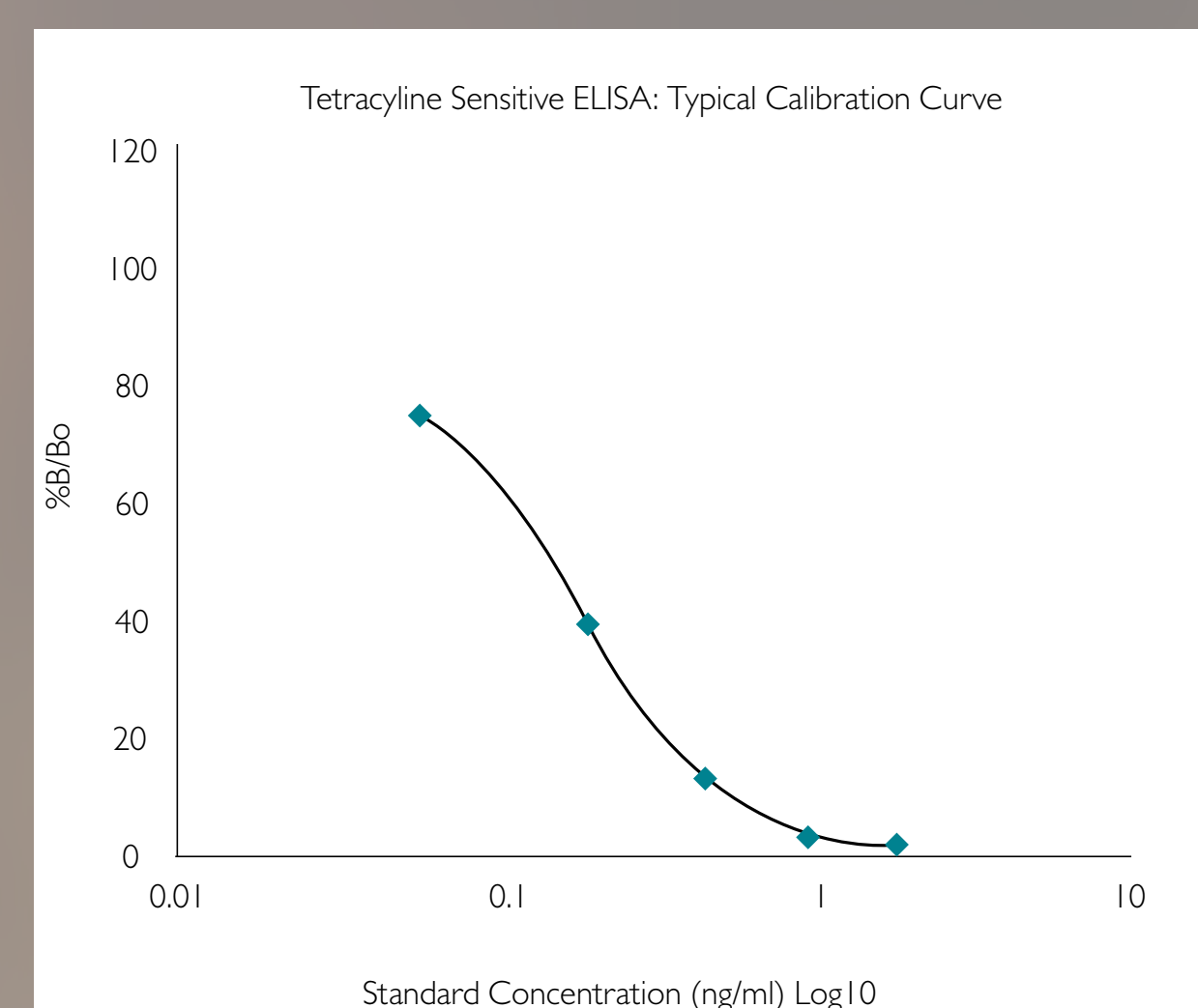
Analyte	Recovery (%)			
	Level 1	Level 2	Level 3	
Tetracycline	86	89	75	Sample 1
	110	119	111	Sample 2

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#### Precision

Analyte	Intra Assay Precision							
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Control 1	Control 2
Tetracycline	CV (%)							
	1.6	1.8	3.3	2.2	5.5	6.0	4.0	5.8

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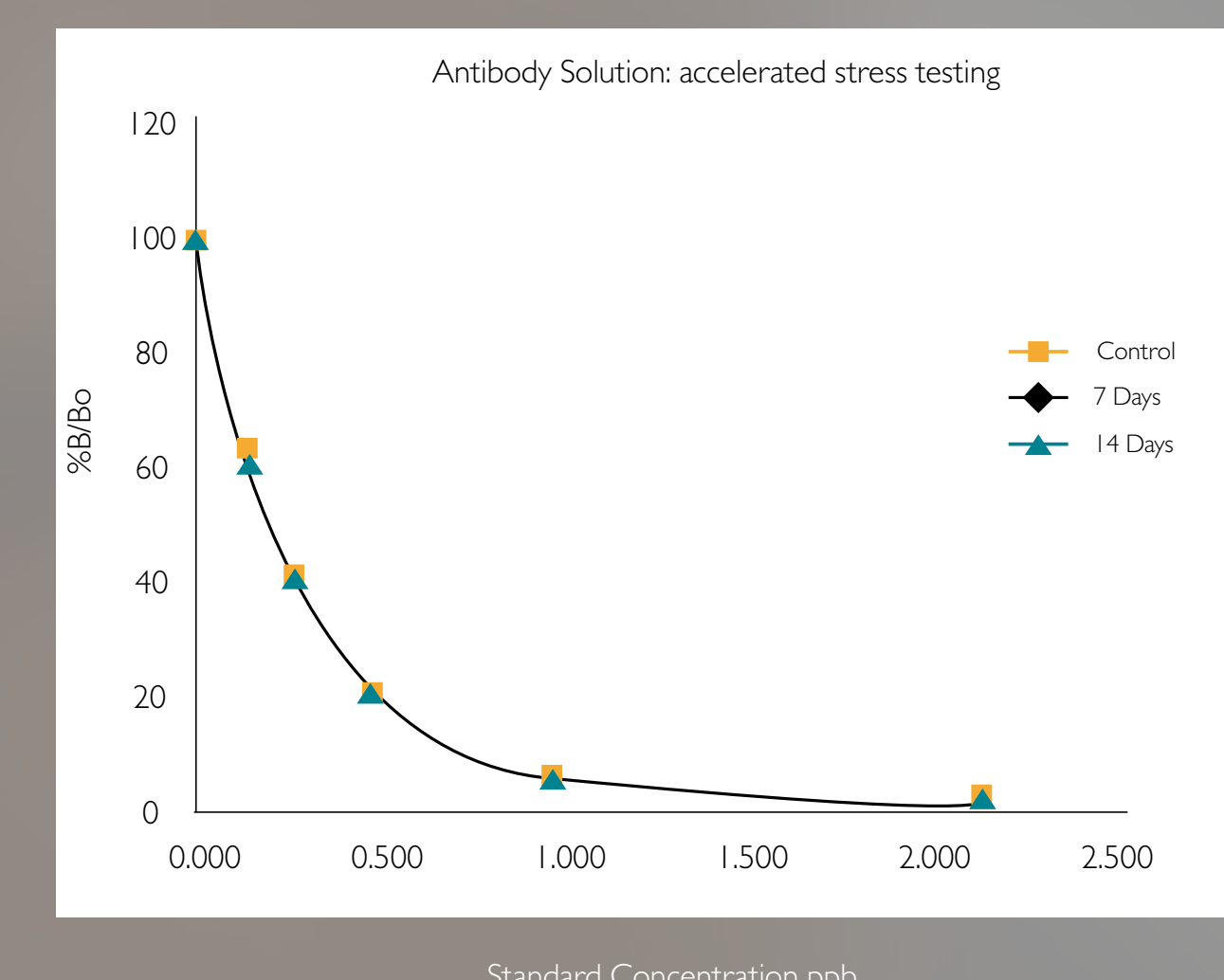


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#### Stability

Predicted shelf life stability: 2 years.

Example:



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Tetracycline Sensitive ELISA TCS101.17.A

### CONCLUSION

- The results indicate that this ELISA is applicable to the detection of tetracycline in honey with a LOD=2 ppb (tetracycline equivalence)
- Total assay time 1 hour
- A standard series, spiking material and ready to use conjugate are included

- Shelf life stability: 2 years
- This represents a convenient and rapid analytical tool for the screening of tetracycline residues in honey following a minimal sample preparation