

## DEVELOPMENT OF A HIGHLY SPECIFIC ELISA FOR THE MEASUREMENT OF **FLUMEQUINE**

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### Introduction

Flumequine is a first generation fluoroquinolone antimicrobial agent with a broad spectrum of activity against aerobic gram-negative bacteria. It belongs to the group of quinolones which are synthetic antibiotics. It is used for the treatment of tract infections in domestic animals. The presence of flumequine in foods and the contribution to bacterial resistance has become of increasing concern. Methods to detect flumequine residues are relevant for monitoring and regulatory purposes.

We report the development of a highly specific and quantitative **ELISA** for the determination of **flumequine** in honey samples, which is of value for the effective screening of this compound.

### Methodology

#### Sample Preparation

The sample can be prepared in two ways:

##### Rapid dilution method:

- Addition of assay buffer (37°C) to 1g of a honey sample
- After rolling, dilution and filtration

The sample is now ready for application to the microtitre plate.

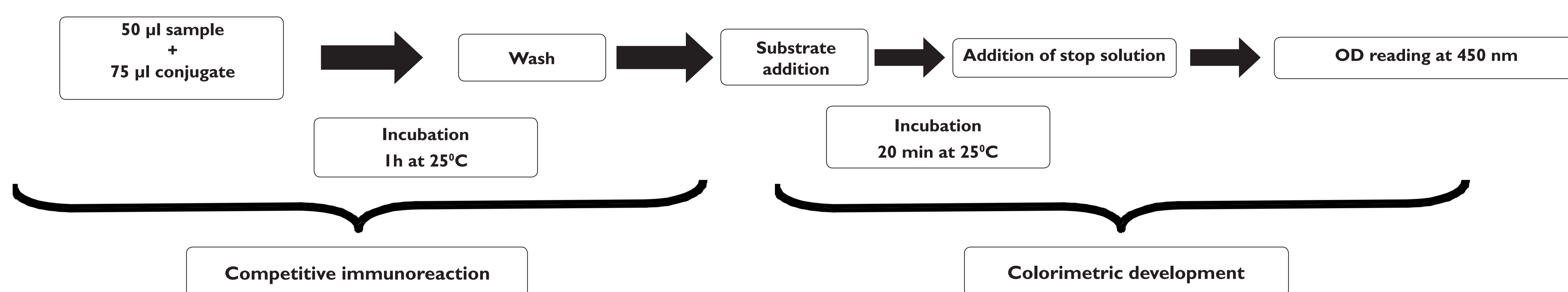
##### Extraction method:

- Addition of assay buffer (37°C) to 1g of a honey sample
- Vortexing and addition of ethylacetate
- Vortexing, rolling and centrifugation
- Elution and evaporation to dryness of the upper organic layer
- After resuspension in assay buffer and vortexing

The sample is now ready for application to the microtitre plate.

#### Competitive ELISA

In-house made capture antibody was immobilised and stabilised on 96 well microtitre plates. The flumequine assay is based on a competitive reaction where any free analyte contained in the standards/samples competes for binding sites of the capture antibody with horseradish peroxidase labelled conjugate. Following the incubation and washing steps, enzyme substrate is added. Measurement of the optical density is carried out at 450nm once the colour reaction is stopped producing a colour change from blue to yellow. Colour intensity is inversely proportional to the concentration of the analyte present.



The analytical performance of the developed ELISA kit (FQ3460, Randox Laboratories, Crumlin, UK) was assessed.

#### Analytical parameters

##### • Limit of Detection (LOD):

LOD was defined as mean concentration of negative samples + 3SD.

##### • Specificity/Cross-reactivity:

the specificity, expressed as % cross-reactivity (%CR) was calculated as follows:

$$\%CR = [IC50 (Flumequine) / IC50 (Cross-reactant)] \times 100$$

The IC50 for each analyte was calculated by taking 50% of the optical density (OD) from the zero calibrator and reading this OD value from the x-axis (concentration in ng/ml) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.

##### Precision

Intra-assay precision (n=12) was determined from the mean results corresponding to six different concentration levels within the same run and was expressed as %CV.

##### Recovery

A negative sample was spiked for a range of concentration levels. Results were expressed as % recovery.



### Results

#### Sensitivity

	Rapid Dilution Method	Extraction Method
Limit of Detection	7.02 ng/g*	4.87 ng/g
Calibration Range	0-20 ng/ml	

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\*Results include assay dilution factor (x10)

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

Analyte	% CR
Flumequine	100
Cinoxacin	<1.83
Ciprofloxacin	<1.83
Danofloxacin	<1.83
Difloxacin	<1.83
Enoxacin	<1.83
Enrofloxacin	<1.75
Marbofloxacin	<1.75
Norfloxacin	<1.75
Ofloxacin	<1.75
Orbifloxacin	<1.75
Oxolonic acid	<1.60
Pipemidic acid	<1.60
Sarafloxacin	<1.60
Nalidixic acid	<1.60

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#### Precision

Analyte	Intra-assay precision (n=12)					
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
Flumequine	2.9	1.2	2.2	1.6	2.9	3.1

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#### Recovery in honey (rapid dilution method)

Analyte	% Recovery (n=20)		
	Level 1	Level 2	Level 3
Flumequine	125.7	117	108.6

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### Conclusion

- Data indicate that the developed competitive ELISA detects specifically flumequine with LOD 7.02 ng/g after rapid dilution method or LOD 4.87 ng/g after simple sample extraction. Intra precision values are typically %CV≤3% over a range of concentration levels.

- Once applied to the microtitre plate 40 samples can be screened in 90min.

- The developed ELISA represents a valuable and convenient analytical tool that can be used for the in vitro quantitative determination of flumequine in honey samples.