

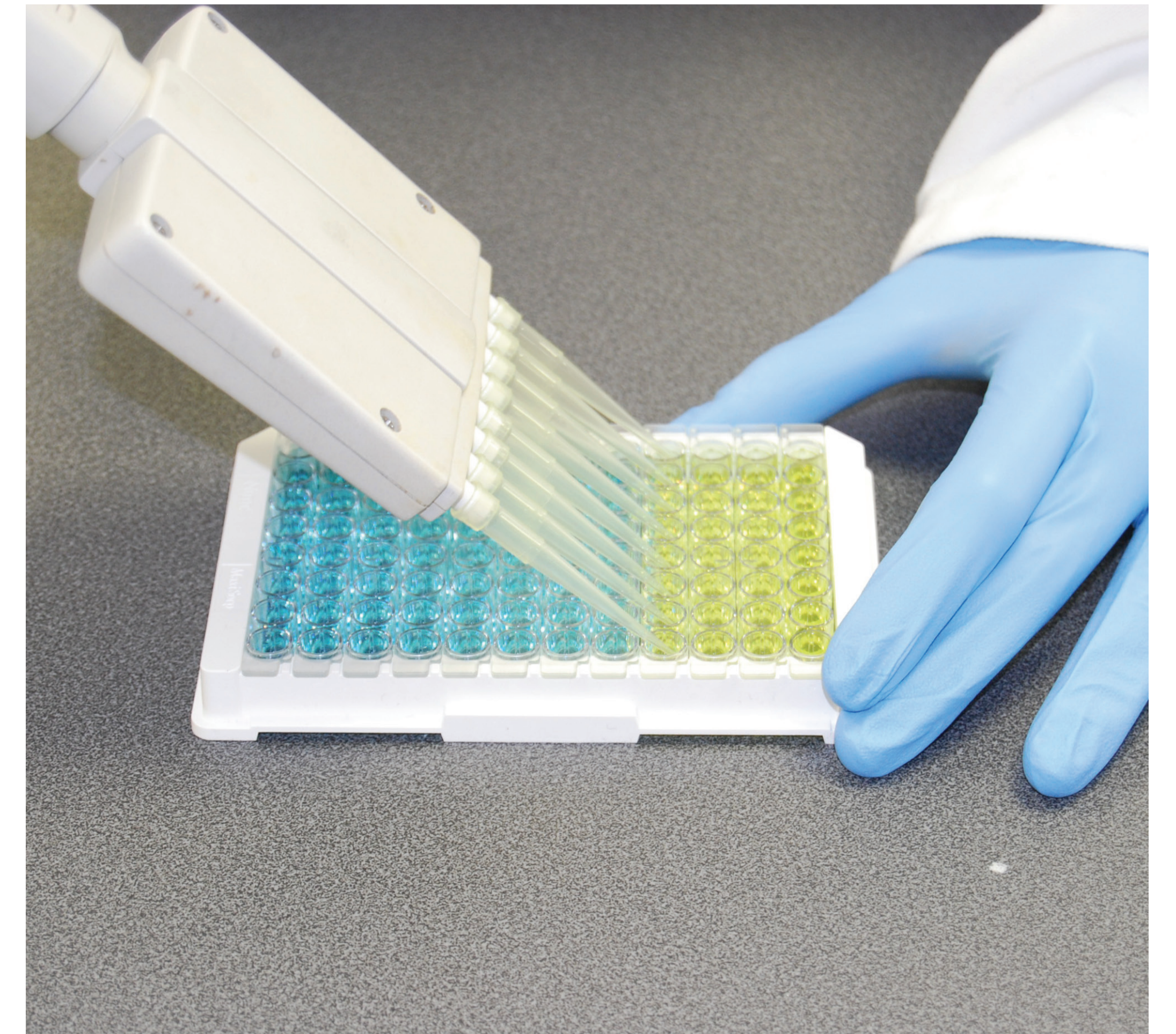
DEVELOPMENT OF A HIGHLY SPECIFIC ELISA FOR THE MEASUREMENT OF SULPHAMETHOXAZOLE.

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Introduction

Sulphamethoxazole is a bacteriostatic sulphonamide and is used in veterinary medicine and in feed. Antibiotics are used worldwide in apiculture to treat or prevent bacterial bee diseases.^(1,2) However antibiotic drug residues in honey pose a potential risk to human health. The European Union has set maximum residue limits (MRLs) for sulphonamides in muscle, fat, liver and kidney from all food producing species, and milk from bovine, ovine and caprine species, but no MRLs have been established for honey, thus the use of sulphonamides in this matrix is not accepted. The availability of rapid and reliable analytical methods for the specific detection of this compound in test samples is of interest for monitoring and regulatory purposes.

We report the development of a highly specific competitive ELISA for the detection of sulphamethoxazole in honey.



Methodology

Sample Preparation:

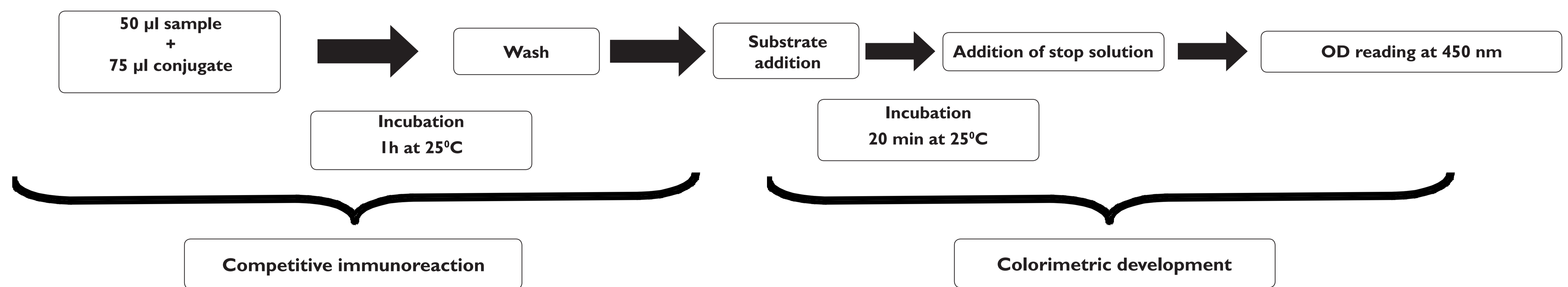
Addition of diluted wash buffer (37°C) to 1g of a honey sample. After rolling, filtration and dilution, the sample is ready for application onto the microtitre plate.

Competitive ELISA

In-house made capture antibody was immobilised and stabilised on 96 well microtitre plates. The sulphamethoxazole 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.

Methodology



The analytical performance of the developed ELISA kit (SZ3471, 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:

$$\%CR = \frac{[IC50 \text{ (Sulphamethoxazole)}]}{[IC50 \text{ (Cross-reactant)}]} \times 100$$

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 honey sample was spiked for a range of concentration levels and further assessed. Results were expressed as % recovery.

Results

Limit of Detection

Limit of Detection* 4.77 ng/g

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*Results include assay dilution factor (x20)

Specificity/Cross-reactivity (CR)

Analyte	% CR
Sulphamethoxazole	100
Sulphaphenazole	<0.576
Sulphaquinoxaline	<0.576
Sulphamonomethoxine	<0.572
Sulphatroxazole	<0.572
Sulphamoxole	<0.572
Sulphanilamide	<0.572
Sulphanitran	<0.572
Sulphacetamide	<0.547
Sulphaguanidine	<0.547
Dapsone	<0.547

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Precision

Intra-assay precision (n=12)

Analyte	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
	%CV	%CV	%CV	%CV	%CV	%CV
Sulphamethoxazole	3.1	4.0	4.0	3.1	3.3	4.2

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Recovery in honey

% Recovery (n=20)

Analyte	Level 1	Level 2	Level 3
Sulphamethoxazole	93.8	117.1	131.2

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Conclusion

- Data indicate that the developed competitive ELISA detects specifically sulphamethoxazole in honey samples with LOD 4.77ng/g. Recovery ranges from 93.8-131.2%. Intra precision values were typically %CV≤4% over a range of concentration levels.

- This assay uses a very simple sample preparation.

- 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 determination of sulphamethoxazole in test samples.

References:

- Hansen H., Brodsgaard C.J., American foulbrood: a review of its biology, diagnosis and control. *Bee World*, 1999; 80: 5-23.
- Williams D. L., A veterinary approach to the European honey bee (*Apis mellifera*). *The Veterinary Journal*, 2000; 160: 61-73.