RAPID SCREENING OF STREPTOMYCIN/DIHYDROSTREPTOMYCIN IN HONEY WITH AN ELISA KIT

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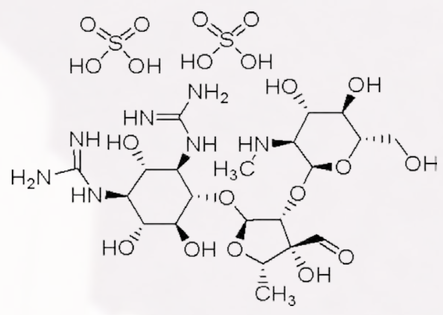
Introduction

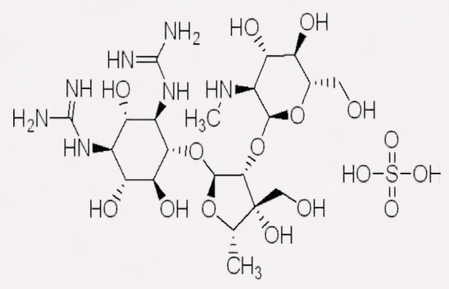
Aminoglycosides, such as streptomycin/dihydrostreptomycin, are mainly used to treat bacterial infections and residues have been found in honey samples worldwide. Regarding the European legislation, no maximum residue limits are fixed for this class of antibiotics in honey, therefore their use is not accepted. For monitoring and regulatory purposes, the use of simple and rapid methods enabling the sensitive detection of these antibiotics in honey is advantageous in test settings.

We report the analytical performance evaluation of an ELISA kit for the rapid screening of streptomycin/dihydrostreptomycin in honey after simple sample preparation

Chemical structures



Streptomycin



Dihydrostreptomycin

Methodology

Principle

The assay is based on the competition between free analyte present in calibrator/sample and horseradish peroxidase labelled conjugate for capture antibody-binding sites. Measurement is carried out using absorbance at 450nm, which is inversely proportional to the concentration of analyte. The assay kit STP3468 (Randox Laboratories Ltd., Crumlin, UK) was used following manufacturer's instructions.

Honey sample preparation

Addition of diluted diluent/wash buffer (37°C) to 1g of a honey sample. After rolling (10 minutes) and filtration the sample is ready for application to the microtitre plate.

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 (Streptomycin)/IC50 (Cross-reactant)] x 100

The half maximal inhibitory concentration (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 ppb) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.

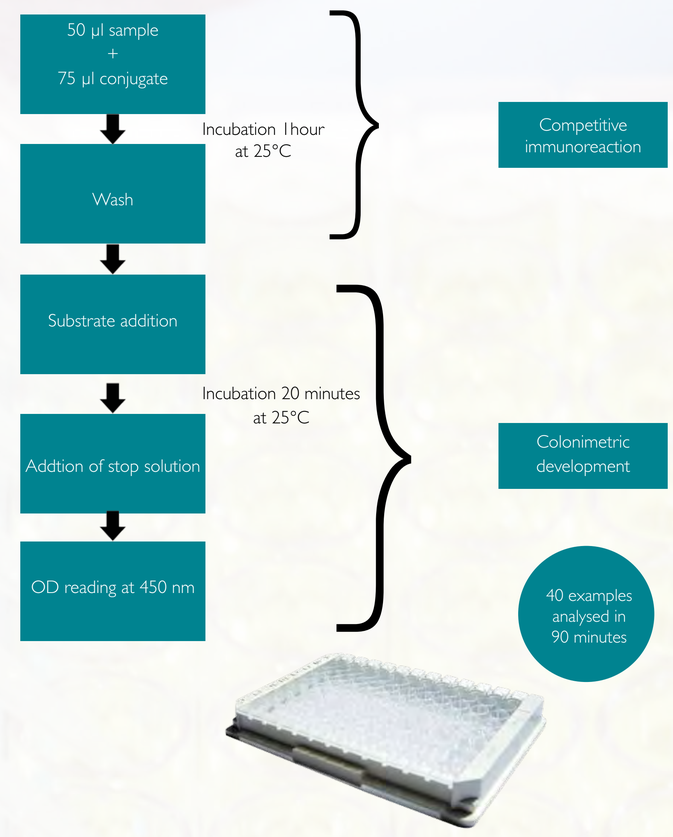
Precision

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

Recovery

Commercially available positive honey samples were examined along with a negative sample spiked for a range of concentration levels.

Experimental procedure outline



Results

Limit of Detection (LOD)

Limit of detection*	6.4ppb

09/041/SM

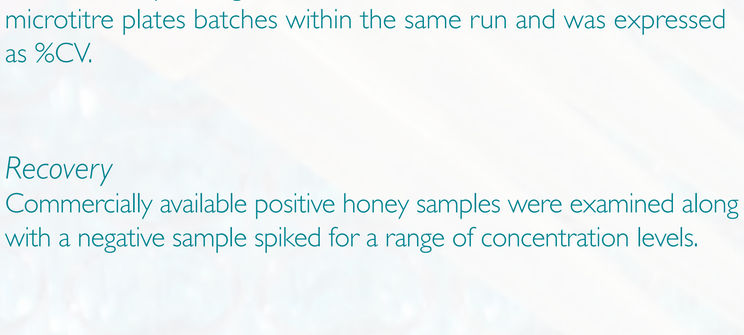
*The immunoassay is standardised against streptomycin

Specificity/Cross-reactivity (CR)

Analyte	%CR
Streptomycin	**100**
Dihydrostreptomycin	106
Gentamicin	< 0.01
Kanamycin	< 0.01
Neomycin	< 0.01
Spectinomycin	< 0.01

09/043/SM

Intra-assay precision



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Recovery

09/044/SM

Conclusions

- Results indicate that this ELISA kit can be used for the rapid screening of streptomycin and dihydrostreptomycin in honey samples.
- Simple sample preparation procedure.
- This assay has a capability to analyse 40 samples in 90 minutes and is applicable to other matrix types (tissue, urine, milk).

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