

## DEVELOPMENT OF A SENSITIVE ELISA FOR THE DETERMINATION OF STREPTOMYCIN/DIHYDROSTREPTOMYCIN IN HONEY.

N. Kane, R.I. McConnell, A. Tohill, S.P. FitzGerald.  
Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim BT29 4QY United Kingdom

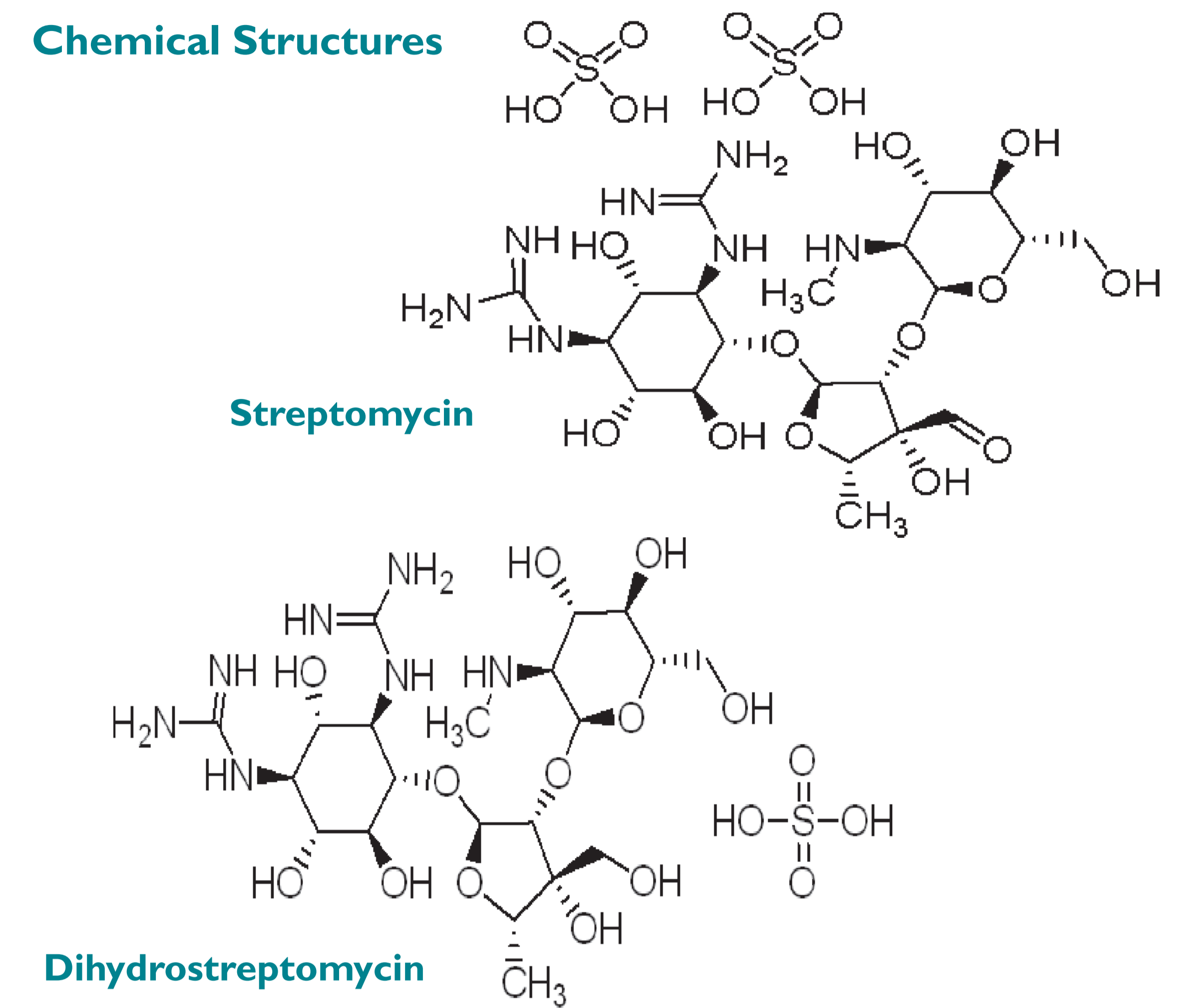
### Introduction

In veterinary medicine streptomycin is one of the first line antibiotics used mainly to treat infections caused by aerobic gram-negative bacteria although they also act synergistically against some gram-positive bacteria<sup>(1-3)</sup> Streptomycin and dihydrostreptomycin are aminoglycosides with related structures. Aminoglycosides have also been used as growth promoters in food producing animals and there has been controversy surrounding this due to the potential for the development of aminoglycoside resistant pathogens.<sup>(4)</sup> Honey is generally accepted as being a natural food product giving health benefits to the consumer. However, over the last few years traces of streptomycin

have been found in a growing number of honey samples worldwide. Contamination of honey may also occur through antimicrobial treatment of plants.<sup>(5)</sup> Regarding the European legislation, no maximum residue limits are fixed for antibiotics in honey, therefore their use is not accepted.

The development of simple and sensitive methods for the detection of **streptomycin** and **dihydrostreptomycin** in honey are of value for monitoring and regulatory purposes. We report the development of a sensitive competitive **ELISA** for the screening of these compounds in honey after simple sample preparation.

### Chemical Structures



### Methodology

#### Sample Preparation:

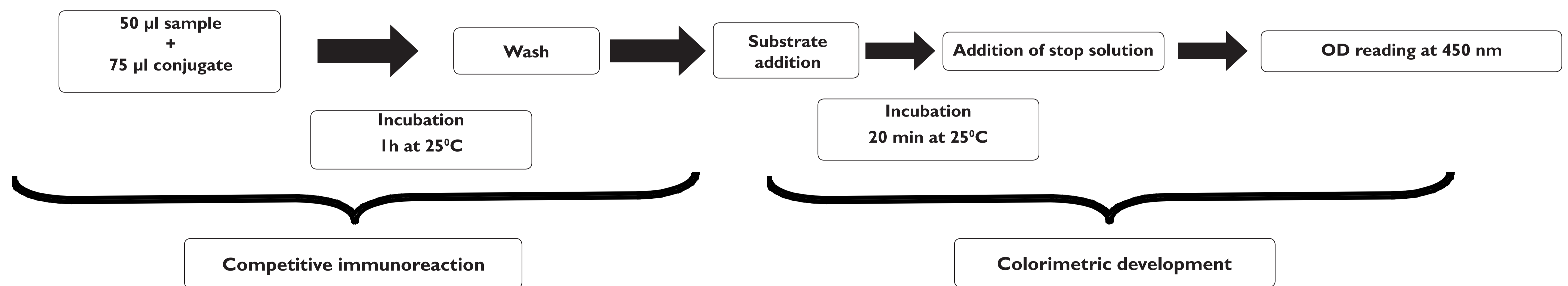
Addition of diluted wash buffer (37°C) to 1g of a honey sample. After rolling and filtration the sample is ready for application onto the microtitre plate.

#### Competitive ELISA

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



### 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:

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

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 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.

#### Reagent stability

Microtitre plate, calibrator stock solution and conjugate stabilities were tested by storing the reagents at 37°C for 14 days, the performance was then compared to material stored at +2-+8°C.

### Results

#### Limit of Detection

Limit of Detection\* 6.4ppb

0904105M  
\*The immunoassay is standardised against streptomycin.

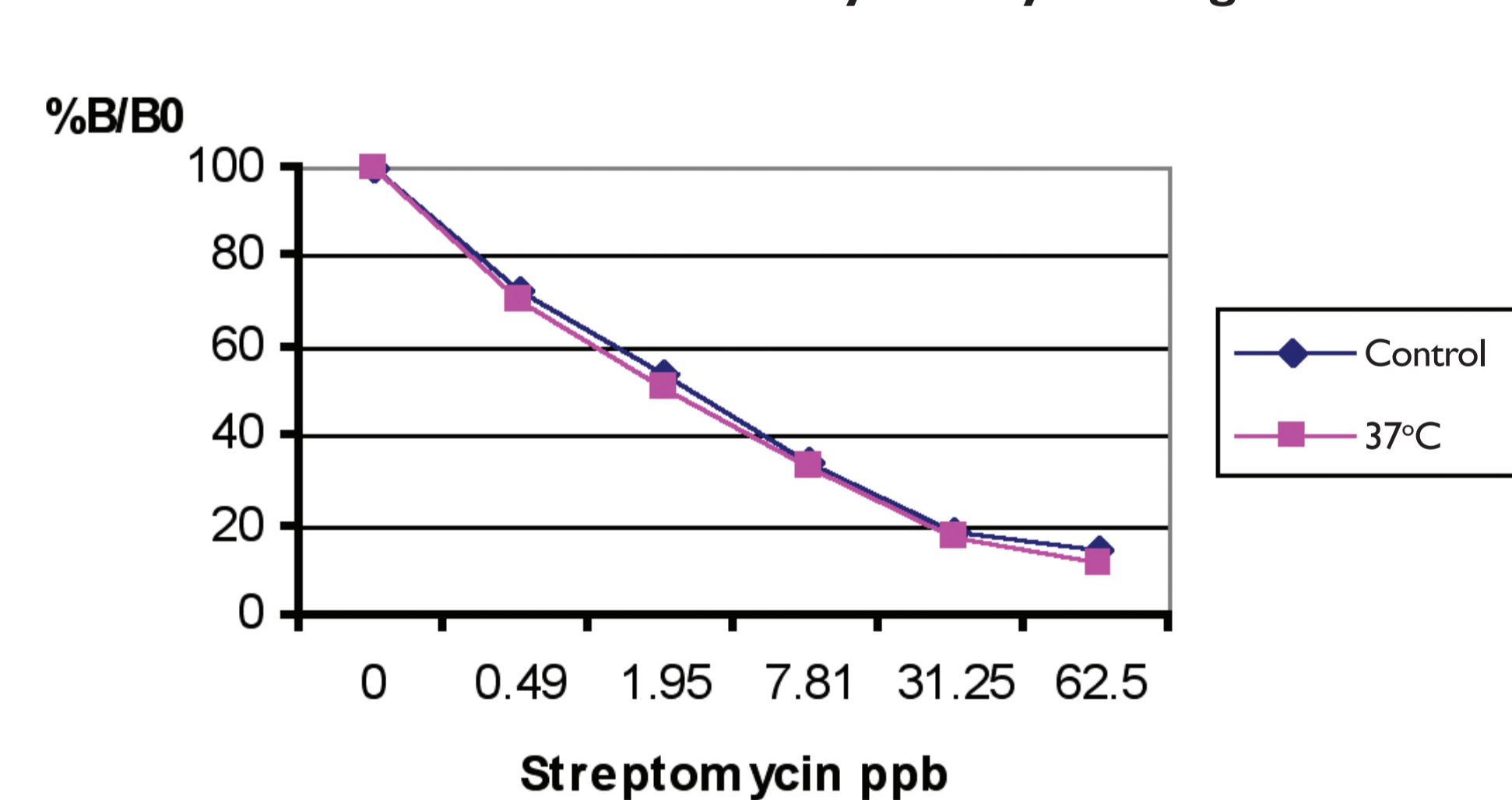
#### Specificity/Cross-reactivity (CR)

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

0904105M

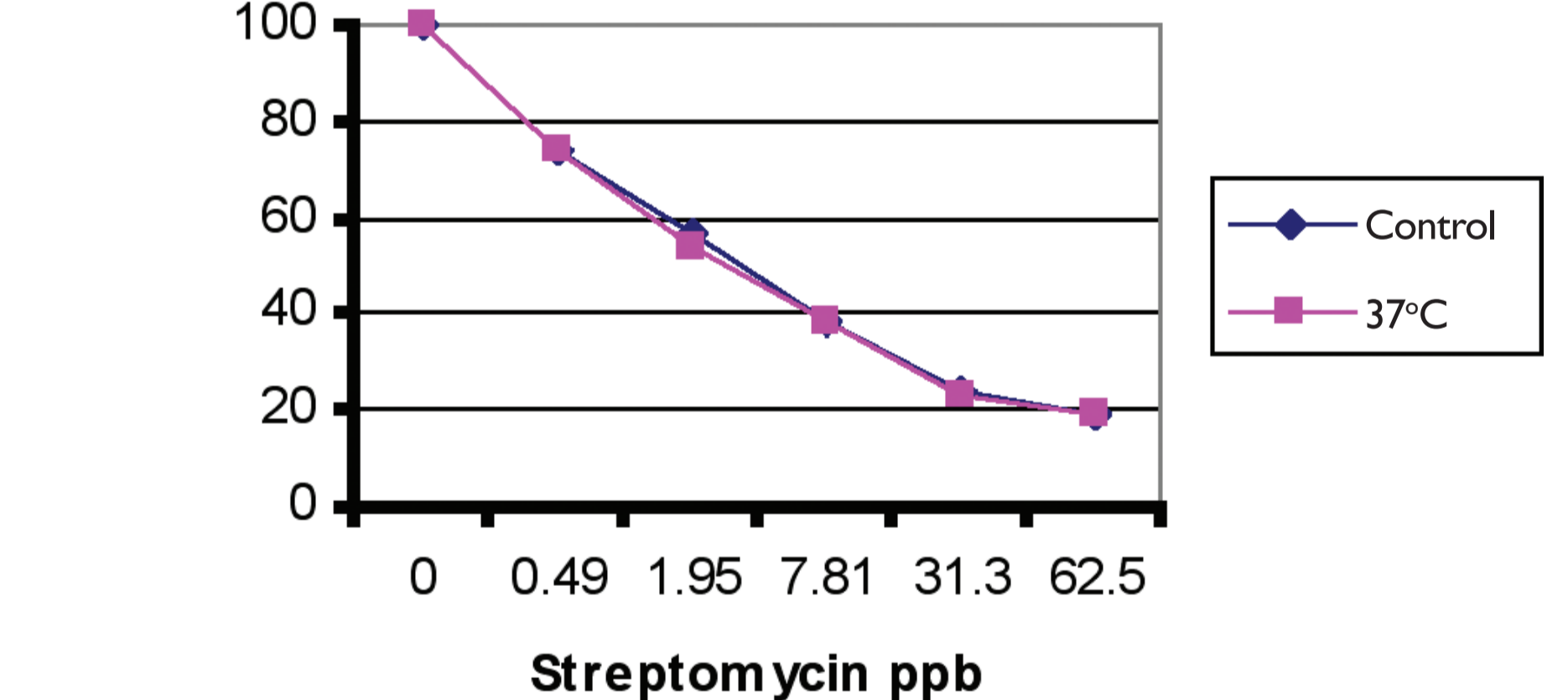
#### Reagent stability

##### Microtitre Plate Stability: 14 days storage at 37°C



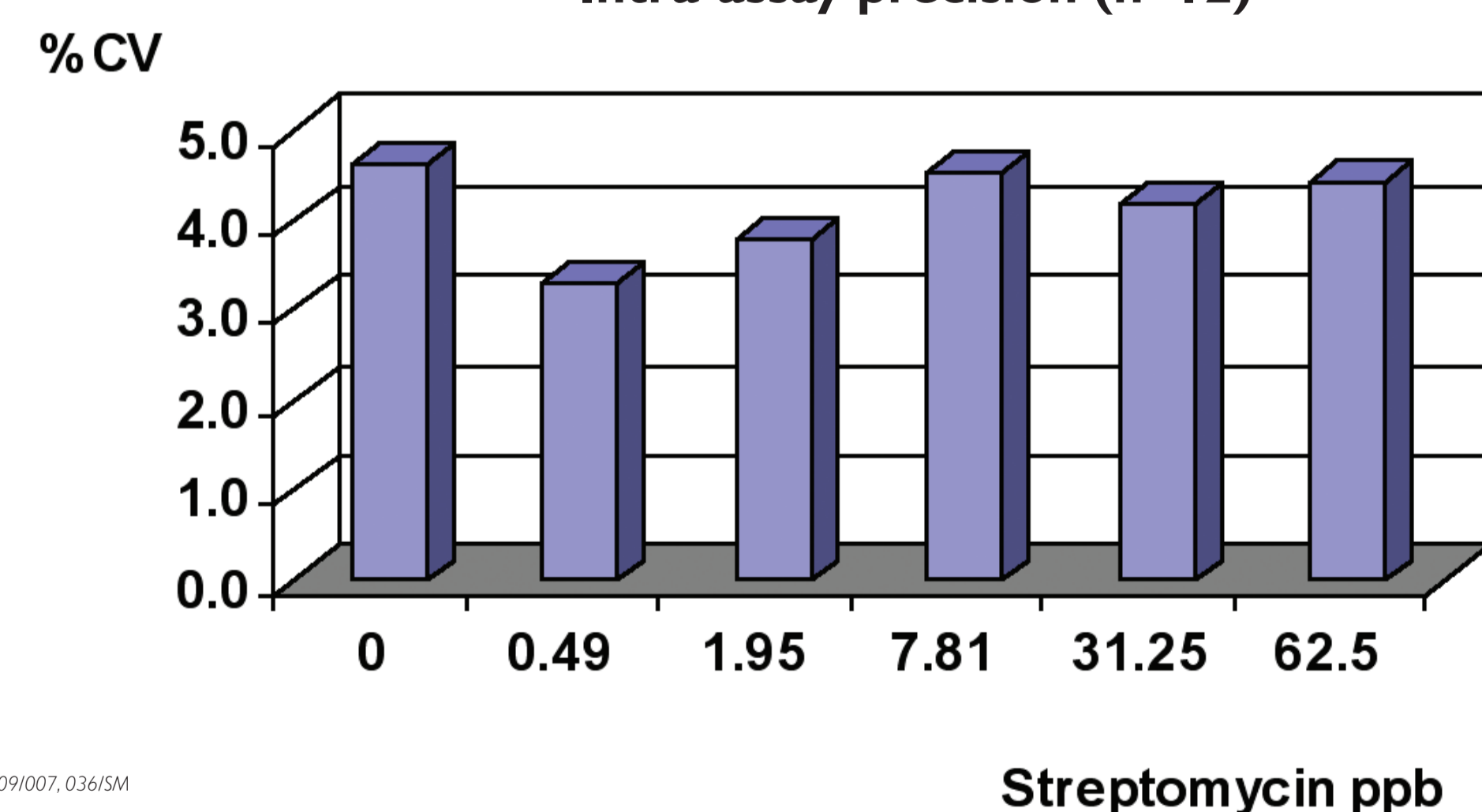
#### Reagent stability

##### Conjugate Stability: 14 days storage at 37°C



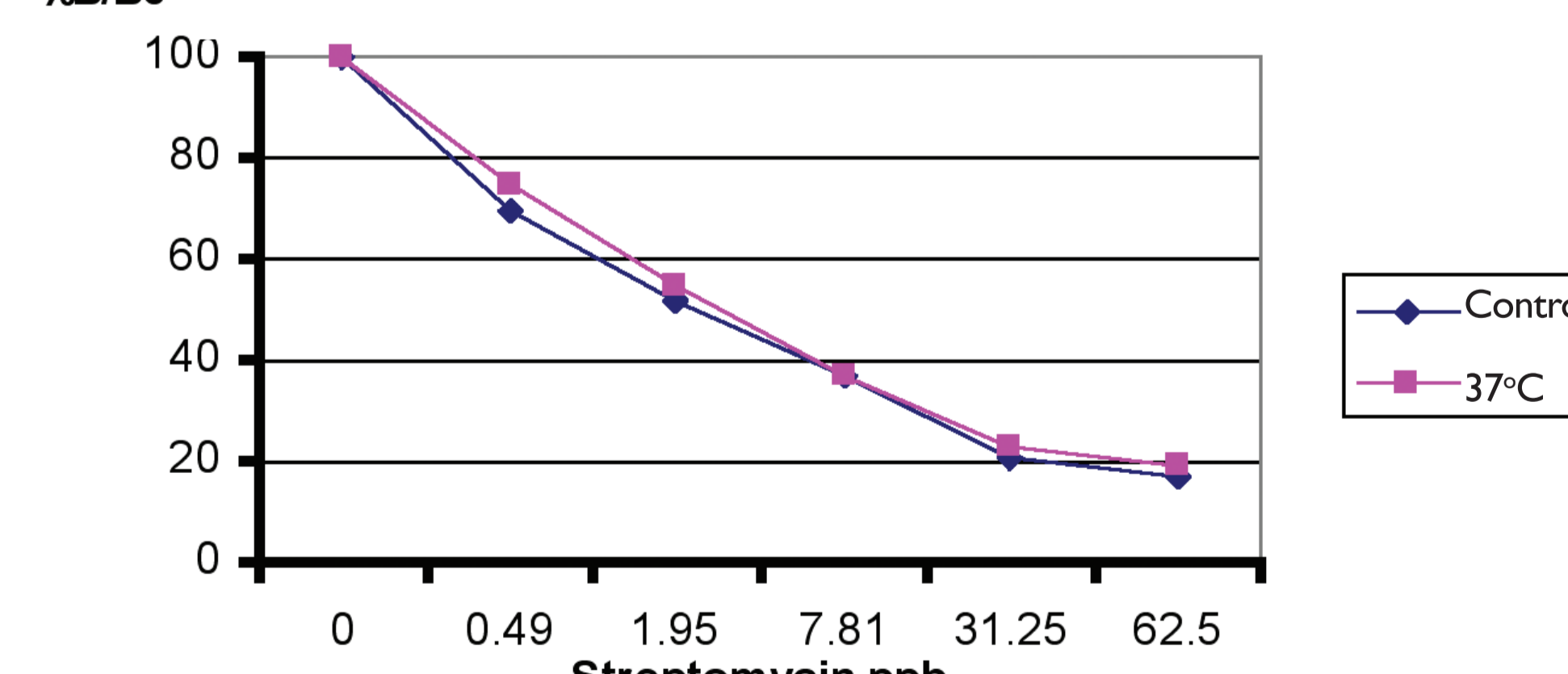
#### Intra-assay precision

##### Streptomycin-dihydrostreptomycin ELISA Intra-assay precision (n=12)



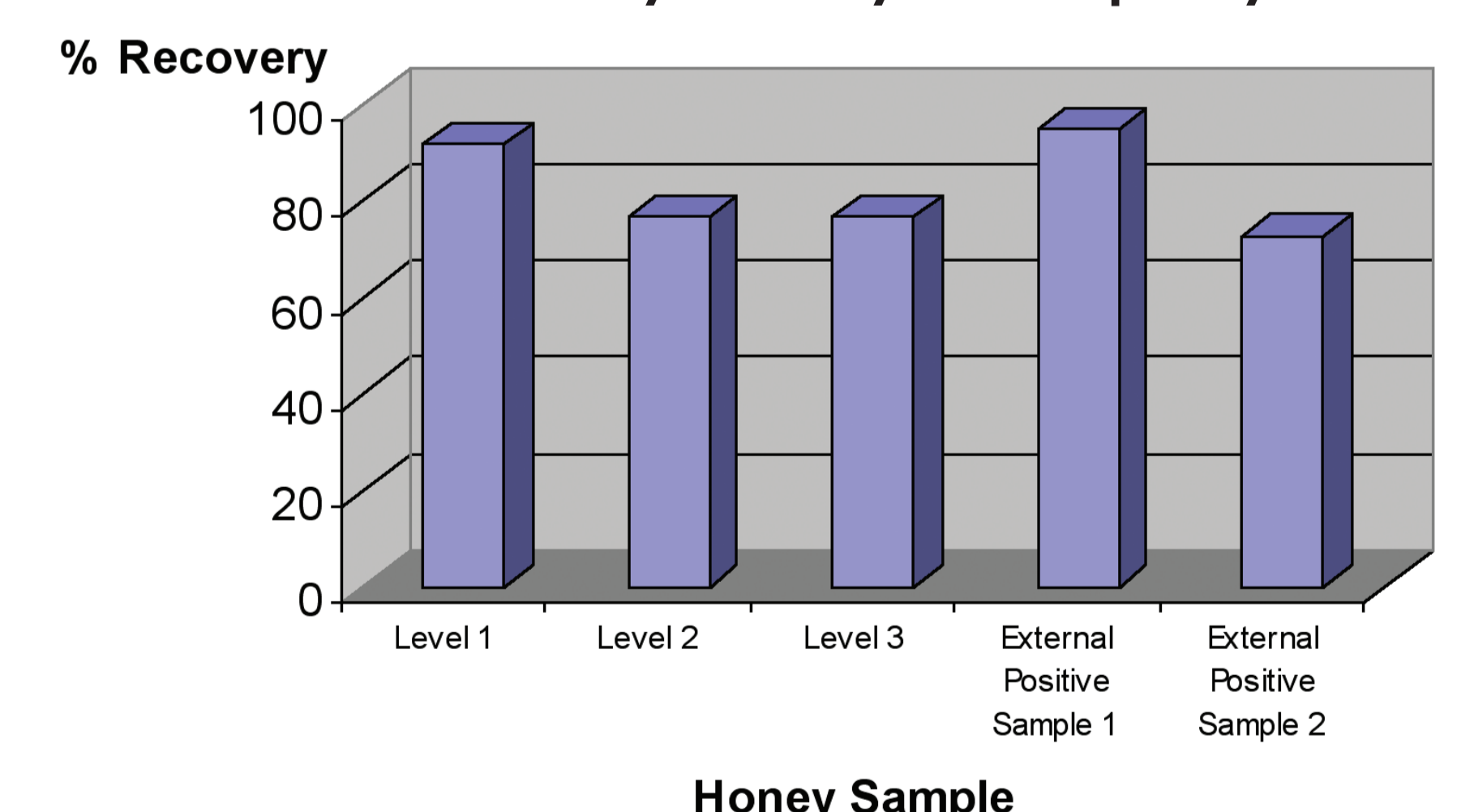
#### Reagent stability

##### Calibrator Stock Solution Stability: 14 days storage at 37°C



#### Recovery

##### Recovery in honey for Streptomycin



### Conclusion

- Data indicate that the developed competitive **ELISA** detects **streptomycin and dihydrostreptomycin in honey samples** with LOD 6.4ppb for streptomycin. Recovery ranges from 77-100% when commercially available positive honey samples and spiked samples were examined. Intra-assay precision values of  $\leq 5\%$  were determined across 3 batches over a range of concentration values.

- This assay uses a very simple sample preparation and does not require the use of many additional consumables.

- The shelf life of the precoated microtitre plate, calibrator stock solution and conjugate was assigned as 2 years at +2-+8°C.

- 40 samples can be screened in 90 minutes.

This ELISA is now commercially available  
(Cat No. STP3468, Randox Laboratories, Crumlin, UK)

### References:

- The Merck Manual of Diagnosis and Therapy, Section 13: Infectious Diseases, Chapter 153: Antibacterial Drugs, Aminoglycosides.
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- Gonzalez, L.S. and Spencer, J.P. Aminoglycosides: A Practical Review. *American Family Physician*; 1998, **58(8)**: 1811-20.
- Hughes, P. and Heritage, J. Antibiotic growth promoters in food animals. *FAO Animal Production and Health Papers*, 2004, **160**: 129-52
- Backman, C. and Tornqvist, P. Automated Determination of Antimicrobial Drug Residues in Honey, *EuroResidue IV*, 2000, **1**: 193-195.