

## DEVELOPMENT OF A HIGHLY SENSITIVE POLYCLONAL ANTIBODY FOR THE DETERMINATION OF LIDOCAINE IN BIOLOGICAL FLUIDS

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### Introduction

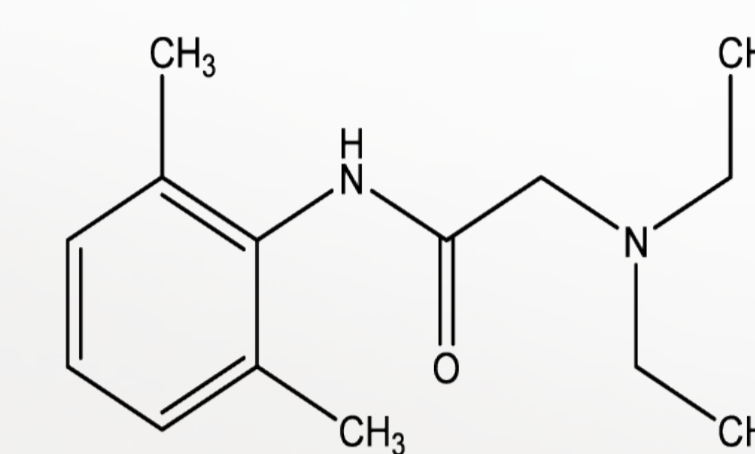
Lidocaine was originally developed as a local anaesthetic. However, it also possesses antiarrhythmic properties, particularly against ventricular arrhythmias and is widely used in the treatment of post-myocardial infarction. The toxic side effects of lidocaine, hypertension, CNS depression and convulsions, appear to be avoidable if the blood levels do not exceed 5µg/ml. The detection of lidocaine in forensic samples can result in significant penalties. Lidocaine is metabolised in the liver by N-deethylation to produce N-desethylidocaine and the di-nor-lidocaine.

For optimum therapeutic management of patients treated with lidocaine, a rapid and specific analytical method is needed that is sensitive enough to measure plasma concentrations of the drug. This need has led to the development of a variety of analytical procedures to determine blood levels of lidocaine.<sup>1-4</sup>

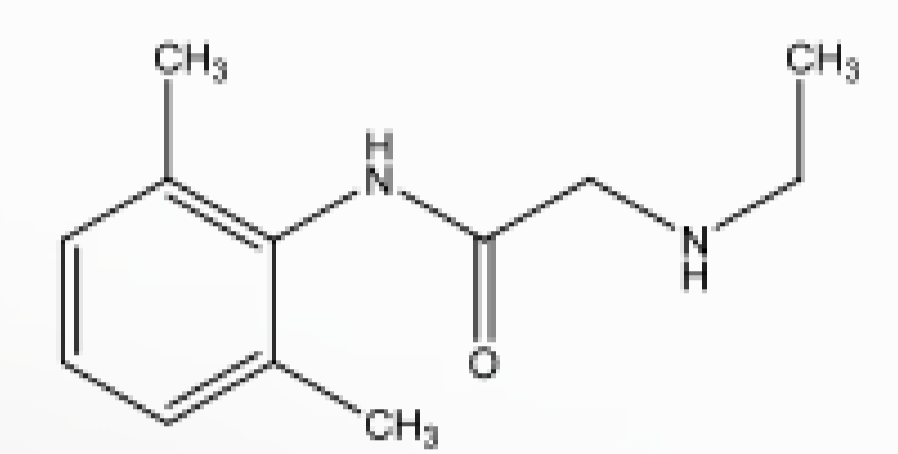
We report the development of a sensitive and specific polyclonal antibody to lidocaine for use in the development of immunoassays for therapeutic drug monitoring applications.

### Chemical structures

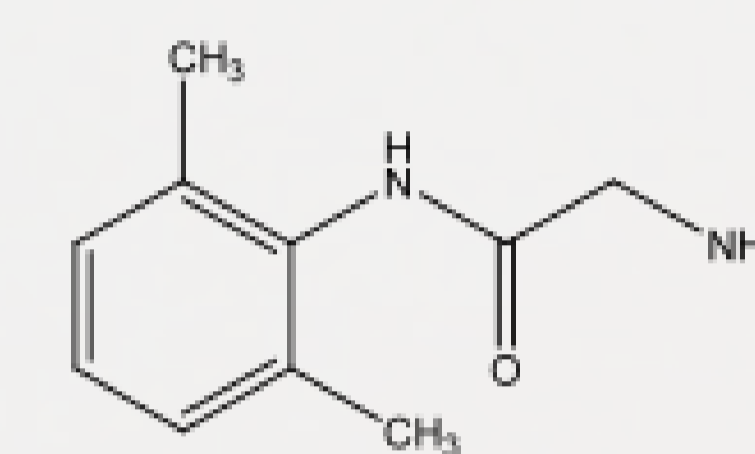
Lidocaine



N-desethylidocaine



Di-nor-lidocaine



### Methodology

A novel hapten to lidocaine was synthesized and conjugated directly to bovine thyroglobulin (BTG) as carrier. The resulting immunogen was administered to adult sheep to generate lidocaine specific polyclonal antiserum. Ig fraction was extracted from the antiserum and evaluated via competitive ELISA.

Absorbance was read at 450nm.

#### Assay evaluation parameters

- The  $IC_{50}$  for each analyte was calculated by taking 50% of the absorbance of the zero calibrator and reading this absorbance value from the x-axis (concentration ng/ml) of the respective calibration curve. This concentration corresponded to the inhibitory concentration that produced 50% inhibition.
- The calibration curves were generated with each of the analytes as standards in the competitive assay. B/B<sub>0</sub> values were calculated where B is the absorbance measured at 450nm for x ng/ml of the analyte and B<sub>0</sub> is the absorbance measured at 450nm in the absence of analyte.
- Specificity: the specificity, expressed as %cross reactivity (%CR) was calculated as follows:  

$$\%CR = [IC_{50}(\text{analyte}) / IC_{50}(\text{cross-reactant})] \times 100$$
- Intra-assay precision was determined from the results of 2 replicates at different concentration levels within the same run. Results were expressed as %CV values.

### Results

Results corresponding to the initial antibody evaluation are presented.

#### Sensitivity and specificity

	Calibration range (ng/ml)	IC <sub>50</sub> (ng/ml)	Specificity %Cross-reactivity
Lidocaine	0-100	2.19	100
Norlidocaine	0-100	>100	<2.2

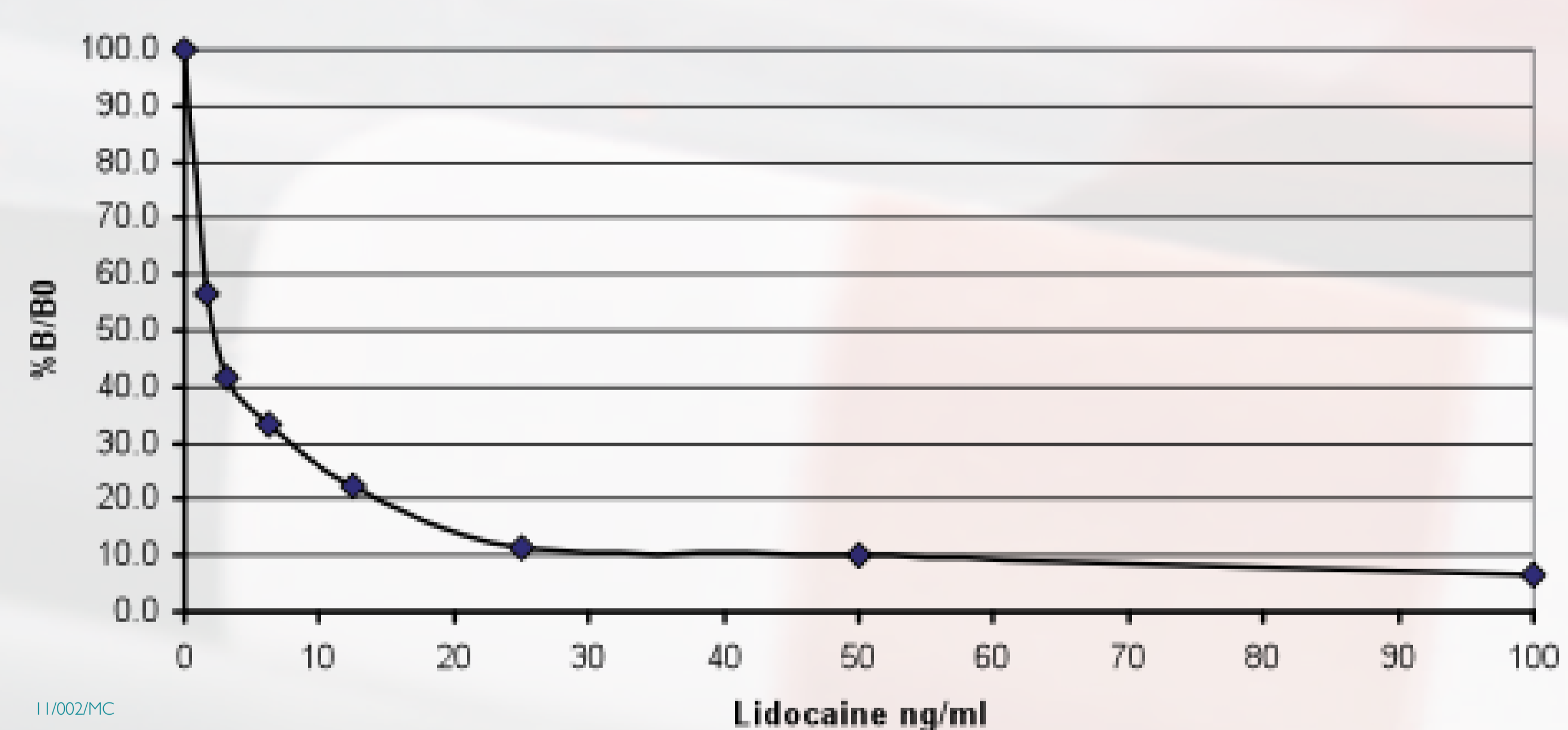
11/002/MC

#### Intra-assay precision

%CV	Lidocaine							
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
	2.1	3.7	7.4	4.3	2.8	6.3	1.7	0.0

11/002/MC

#### Calibration curve for Lidocaine



11/002/MC

### Conclusion

This study reports the development of a highly sensitive and highly specific polyclonal antiserum for the detection of lidocaine.

Initial data indicate that the antibody exhibits 100% specificity for lidocaine and <2.2% cross-reactivity with norlidocaine. The intra-assay precision (n=2x8) expressed as %CV is typically <7.5%.

This antibody is of value for the development of sensitive, specific screening methods for the detection of lidocaine in biological fluids.

#### References

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- Dusci, L.J. and Hacket, L.P., *Clin. Toxicol.* 1979, 14(5): 587-593.
- Lee, K.Y., et al., *J. Chromat.* 1978, 158: 403-410.
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