DEVELOPMENT OF A GENERIC MONOCLONAL ANTIBODY AGAINST BARBITURATES

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Introduction

Barbiturates are a class of compounds derivatised from barbituric acid. They are central nervous system (CNS) depressants and can be used as sedatives, hypnotics, anaesthetics and anti-epileptic drugs. Immunoassays enabling generic determination of barbiturates are of interest to monitor their use/misuse for applications in therapeutic, forensic and toxicology settings.

The aim of this work was to produce a generic monoclonal antibody presenting a broad specificity profile, which will be of value in the development of immunoassays for the detection of barbiturates.

Methodology

Sheep were immunized with the barbituarate conjugated via an amino group to a carrier protein bovine thyroglobulin (BTG). Lymphocytes were collected and fused with heteromyeloma cells. The resulting hybridomas supernatants were screened for the presence of antibody using competitive ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by competitive ELISA.

Assay evaluation parameters

- The IC_{50} for each analyte tested was calculated by taking 50% of the absorbance of the zero calibrator and reading this absorbance 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.
- The calibration curve was generated with the analyte as standard in the competitive ELISA. B/B0 values were calculated, where B is the absorbance measured at 450nm for x ng/ml of the anlyte and B0 is the absorbance measured at 450nm in the absence of analyte.
- Specificity, expressed as % cross-reactivity (%CR), was calculated as follows:
  %CR = $[IC_{50}(\text{phenobarbital}) / IC_{50}(\text{cross-reactant})] \times 100$
- Intra-assay precision was determined from the results of 3 replicates at different concentration levels within the same run. Results were expressed as %CV values.

Results

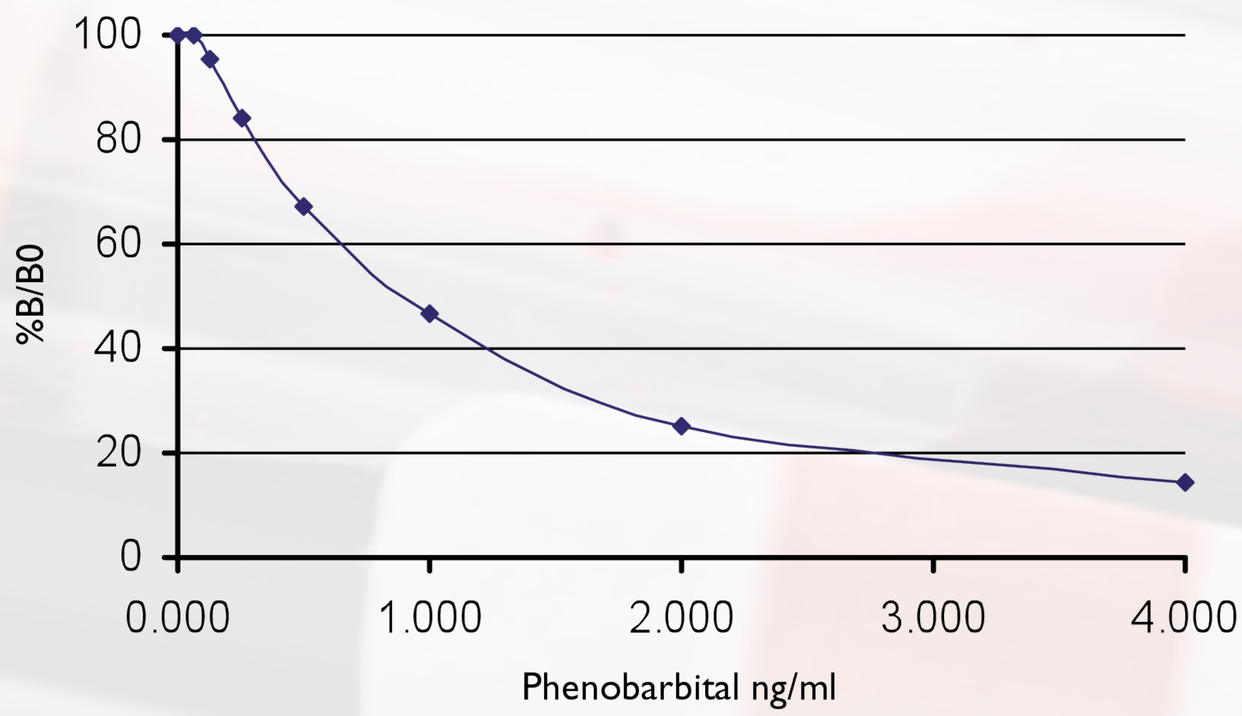
Results corresponding to the initial antibody evaluation are presented.

Sensitivity and specificity

	Calibration range (ng/ml)	IC50 (ng/ml)	%Cross-reactivity
Phenobarbital	**0-4**	**0.868**	**100.0**
Alphenal	0-1	0.136	638.2
Secobarbital	0-1	0.277	313.4
Aprobarbital	0-10	0.385	225.5
Butabarbital	0-40	0.592	97.3
Amobarbital	0-10	1.159	74.9
Pentobarbital	0-10	1.604	54.1
Barbital	0-40	5.960	14.6
Mephobarbital	0-1000	118.760	0.7
Hexobarbital	0-1000	438.650	0.2

11/005/MC

Calibration curve for phenobarbital



Intra-assay precision (n=3 x 8): %CV <5% for phenobarbital at different concentrations

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Conclusion

This generic monoclonal antibody exhibits high sensitivity and specificity for a wide range of barbiturates. This is of value in developing effective immunoassays applicable to therapeutic, forensic and toxicology fields.

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