# Specificity and Selectivity of Novel Anti-Human P450 Antibodies

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## Introduction

*In vitro* metabolism studies are becoming an ever increasing component of the drug discovery and development processes. The data generated enable investigators to make predictions about rates and routes of metabolism, species variation in metabolism and, prior to exposing humans to the novel entities, the potential for drug-drug interactions.

The characterisation of which specific P450(s) metabolises a novel drug is of prime importance in understanding possible drug-drug interactions and potential toxicological end points. Anti-human P450 antibodies are a useful diagnostic tool for these reaction phenotyping studies in human-based *in vitro* systems. As well as aiding in the elucidation of P450 isoforms involved in particular routes of metabolism of novel drugs they may also be used to provide information on the quantitative distribution of P450 isoforms between tissues and spatial distribution within tissues

As with all antibodies, the usefulness of anti-P450 antibodies will be limited by their selectivity and specificity for the target isform. In this study we have examined the selectivity/specificity of anti-CYP1A2, 2A6, 2C8, 2C9, 2D6, 3A4 antibodies obtained from a new commercial source and then used these antibodies to examine the tissue distribution of the relevant P450 isoform in liver, kidney, small intestine and lung. Antibody selectivity/specificity was determined using Bactosomes® which express a single P450 isoform.

## Methods

Microsomes from human liver, lung, kidney and small intestine were prepared by differential centrifugation of the respective tissue homogenate. Bactosomes expressing single P450 isoforms were obtained from CYPEX Ltd. Dundee, UK. Anti-human P450 antibodies (manufactured by Nosan, Japan) were also obtained from CYPEX. Proteins were resolved on 10% acrylamide gels. For Ab selectivity experiments 5 µg of protein was loaded per well and for tissue distribution 10, 20 or 50 µg of protein was loaded per well. Following electrophoresis proteins were transferred to PVDF filters. The PVDF filters were then probed with the respective primary anti-P450 Ab at a dilution of 1 in 10,000 and the secondary antibody (anti-rabbit IgG peroxidase conjugate) at a dilution of 1 in 2,000. The blots were visualised by enhanced chemiluminescence.

## Results

### Selectivity/Specificity

From a visual assessment of the immunoblots shown in Figure 1 the following results were obtained:

- Anti-CYP1A2, 2A6, 2C9, 2D6 antibodies were all found to be very selective and specific for their target P450 isoform with no detectable cross-reactivity with other P450 isoforms
- Anti-CYP2C8 was found to be very selective but not specific for this isoform, with cross-reactivity with CYP2A6, 2C19, 2D6 and 3A5 isoforms evident
- Whilst anti-CYP3A4 was found to be selective for CYP3A isoforms it was found to cross-react with CYP3A5

### Tissue Distribution

From a visual assessment of the immunoblots shown in Figure 2 the following results were obtained:

- The liver was found to contain relatively high expression of all of the isoforms examined
- CYP1A2, 2A6 and 2C9 P450 isoforms were not detected in any of the extrahepatic tissues examined
- Anti CYP2C8 immunoreactive protein was detected in human lung but not in any of the other tissues examined.
  CYP2C8 mRNA has previously been detected in lung tissue by Mace et al (European Journal of Cancer (1994), **34** pp914–920)
- Anti-CYP2D6 immunoreactive protein was detected at all extrahepatic tissues investigate, although to a lesser degree in the lung
- Anti-CYP3A4 immunoreactive protein was detected in small intestine. CYP3A4 is most likely to be found in enterocytes of the small intestine (Krishna & Klotz, Clinical Pharmacokinetic Concepts (1994), **26** pp144–160

1

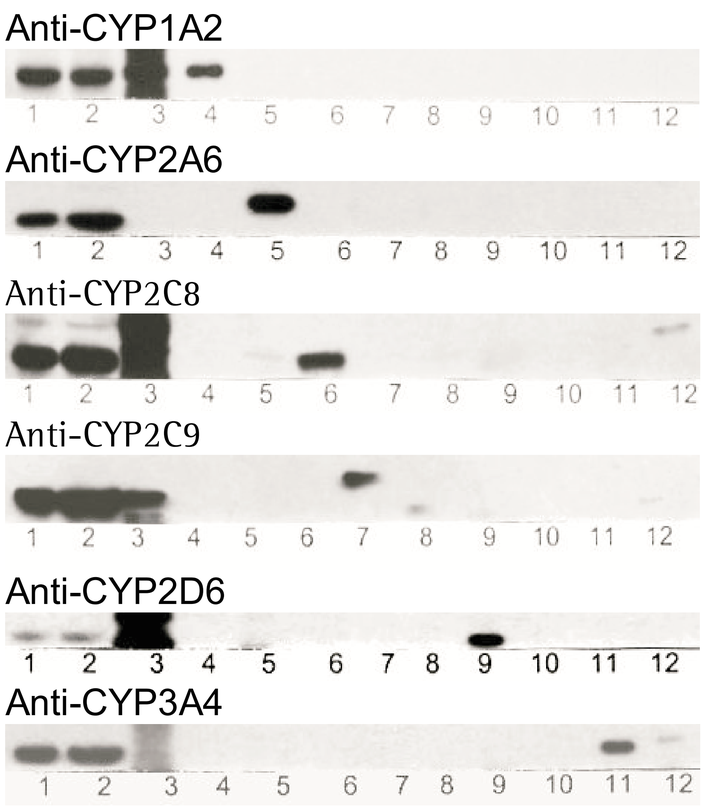


Figure 1: Selectivity of anti-human P450 antibodies against recombinant human P450

Lane numbers as follows: Lane 1and 2 – human liver microsomes, Lane 3 – control bactosomes, Lane 4 – CYP1A2, Lane 5 – CYP2A6, Lane 6 – CYP2C8, Lane 7 – CYP2C9, Lane 8 – CYP2C19, Lane 9 – CYP2D6, Lane 10 – CYP2E1, Lane 11 – CYP3A4, Lane 12 – CYP3A5

2

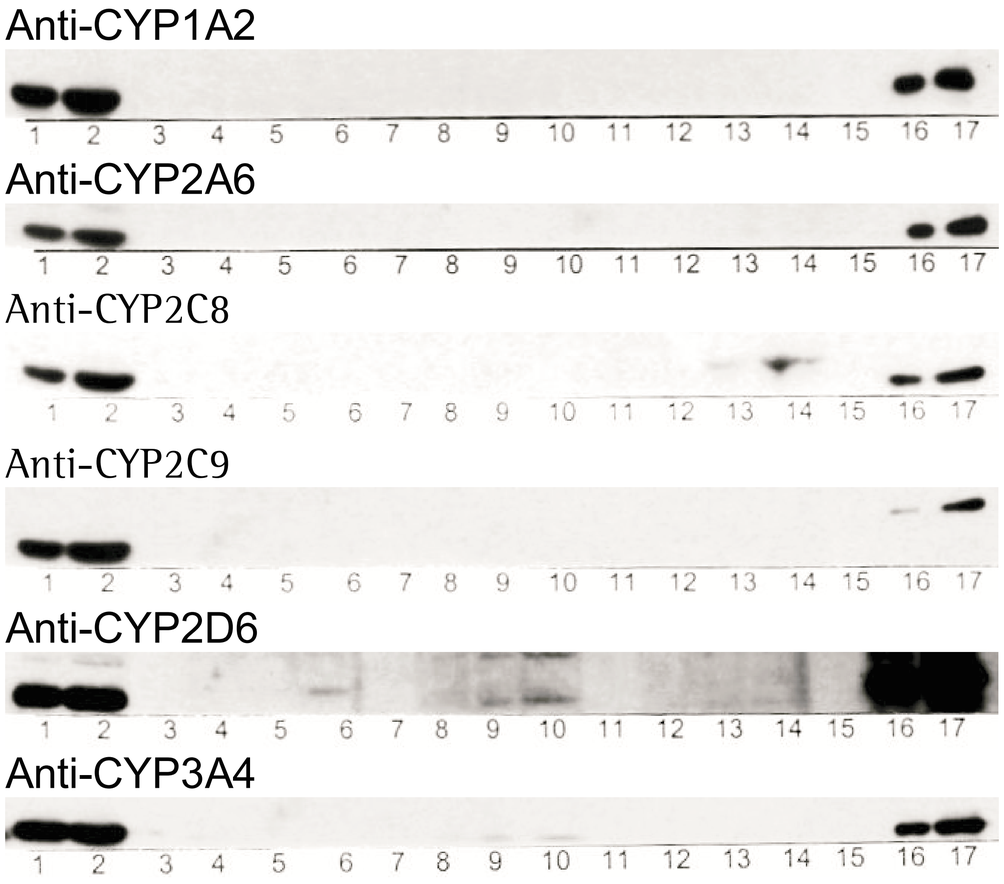


Figure 2: Tissue Distribution of Human P450 – Assessment using Anti-Human P450 Antibodies

Lane numbers as follows: Lane 1 – liver microsomes (10 µg), Lane 2 – liver microsomes (20 µg) Lane 3 – blank, Lane 4 – kidney microsomes (10 µg), Lane 5 – kidney microsomes (20 µg), Lane 6 – kidney microsomes (50 µg), Lane 7 – blank, Lane 8 – gut microsomes (10 µg), Lane 9 – gut microsomes (20 µg), Lane 10 – gut microsomes (50 µg), Lane 11 – blank, Lane 12 – lung microsomes (10 µg), Lane 13 – lung microsomes (20 µg), Lane 14 – lung microsome (50 µg) Lane 15 – blank, Lane 16 – bactosomes (0.25 pmol), Lane 17 – bactosomes (0.5 pmol)

## Conclusion

In conclusion, this novel series of anti-human P450 antibodies are useful diagnostic tools for *in vitro* metabolism studies.

They would seem to offer an advantage over other commercially available antibodies; as the same antibodies have been reported by the suppliers to be suitable for both immuno-inhibition and immuno-blotting. We are currently investigating their potency and specificity for immuno-inhibition and will report our findings separately.