Identification of the P450 enzymes underlying metabolism of a given NME can be utilized to predict not only that compound’s potential to elicit drug-drug interactions but also the impact of polymorphisms on its metabolism. CYP450-GP is in a unique position to offer this service because of our available repertoire of monospecific, well-characterized and proven antibodies against drug-metabolizing P450s. The specificity and inhibitory nature of these antibodies have been established in numerous publications (see Literature section). CYP450-GP’s P450 antibodies are clearly more specific than chemical inhibitors. The results obtained in metabolic immunoinhibition assays performed with liver microsomes can often be confirmed using either purified native or recombinant (heterologously-expressed) P450 enzymes, although examples exist where this is not the case. In such instances, the heterologously-expressed and/or purified P450 protein exhibits a catalytic property different from that found when the same enzyme is embedded in liver endoplasmic reticulum.

A description of our metabolic phenotyping methodology is given in the Protocols section under Antibody Inhibition. An NME of interest (designated herein as GP-123) is known to undergo metabolism by human liver microsomes to a single major product, namely 6-hydroxy GP-123. However, the P450 catalyst underlying this oxidation reaction has not yet been identified. In a typical phenotyping study, GP-123 would be reacted with NADPH-fortified liver microsomes in the presence of fixed amounts of antibodies against the major drug-metabolizing P450s. These same antibodies comprise our CYP ImmunoScreen Kit (Hu-A011). Afterwards, formation of the reaction product 6-hydroxy GP-123 would be analyzed, and rates of product formation vs specific P450 antibody added to assay then derived. In the example shown below, 6-hydroxy GP-123 formation was inhibited by only a single P450 antibody, namely anti-CYP2D6, providing unambiguous evidence that CYP2D6 promotes this GP-123-metabolizing reaction in liver from this subject. Similar studies would then be performed in other subjects to confirm involvement of CYP2D6 in liver microsomal GP-123 6-hydroxylation. An immunotitration experiment performed with anti-CYP2D6 (see Antibody Inhibition) could then be done to reveal the extent of CYP2D6 participation in CYP450-GP-123 metabolism, and whether other P450 enzymes can also hydroxylate this compound.
INHIBITION OF GP-123 6-HYDROXYLATION IN HUMAN LIVER MICROSOMES BY P450 ANTIBODIES

GP-123 6-HYDROXYLATION (% of Control)

ANTIBODY ADDED

PREIMMUNE  ANTI-CYP3A4  ANTI-CYP2D6  ANTI-CYP1A2  ANTI-CYP2C9

0  20  40  60  80  100