

Microsomal and cytosolic scaling factors in dog and human kidney cortex and application for in vitro-in vivo extrapolation of renal metabolic clearance.

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Drug Metabolism & Disposition

Supplementary Material

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Figure S1

Kidney Isolation

Excision only

No perfusion/ wash step before freezing or homogenisation (Dohn and Anders, 1982; Pacifici et al., 1988; Amet et al., 1997; McGurk et al., 1998; Soars et al., 2001; Dai et al., 2004; Tsoutsikos et al., 2004; Al-Jahdari et al., 2006; Ozaki et al., 2015)

Perfused

Saline (0.9% NaCl) (Jakobsson and Cinti, 1973)
Saline with 0.05 M Tricine (pH 8.0) (Okita et al., 1979; Prough et al., 1979; Jakobsson et al., 1982)
Ringer-dextran type solution, then 10% invertose with NaHCO₃ (Wistrand and Knuutila, 1989)

Rinse/ placed in buffer

0.25 M sucrose (Jakobsson, 1974; Aitio and Vainio, 1976)
0.25 M sucrose, 50 mM Tris HCl (pH 7.4) (Litterst et al., 1975)
0.25 M sucrose, 10mM TEA, 1mM EDTA (pH 7.6) (Lash et al., 1998; Cummings et al., 2001)
Saline (0.85 – 0.9% NaCl) (Wise et al., 1984; Nässberger et al., 1987)
1.15% w/ v KCl (Zordoky et al., 2011)
0.1 M K₂PO₄, 0.15M KCl, 1.5mM EDTA (pH 7.4) (Sharer et al., 1992)
50 mM Tris-HCl, 0.15 M KCl, 2 mM EDTA, pH 7.4 *

Homogenisation

Buffer

0.25 M sucrose (Jakobsson and Cinti, 1973; Jakobsson, 1974; Litterst et al., 1975; Aitio and Vainio, 1976; Nässberger et al., 1987; Zordoky et al., 2011)
0.25 M sucrose, 2 mM Tris, pH 7.5 (Tsoutsikos et al., 2004)
0.25 M sucrose, 5 mM HEPES, pH 7.4 (Soars et al., 2001)
0.25 M sucrose, Tris-HCl pH 7.4 (Pacifici et al., 1988)
0.25 M sucrose, 10 mM potassium phosphate, 1 mM EDTA, 1 tablet/ 50 ml protease inhibitor, pH 7.4 (Dai et al., 2004)
0.25 M sucrose, 1 mM NaCO₃, 1 mM EDTA, 1 mM protease inhibitor (Wistrand and Knuutila, 1989)
0.25 M sucrose, 10 mM TEA, 1mM EDTA, pH 7.6 (Lash et al., 1998; Cummings et al., 2001)
0.25 M sucrose, 0.1 M potassium phosphate, pH 7.4 (Dohn and Anders, 1982)
0.05 M Potassium phosphate, 1 mM EDTA, 20% glycerol (Okita et al., 1979; Prough et al., 1979)
3 mM Tris-HCl buffer, pH 7.5 (Jakobsson, 1974)
0.15 M KCl, 1.5 mM EDTA, 0.1 M KH₂PO₄, pH 7.4 (Sharer et al., 1992)
1.15% KCl (Al-Jahdari et al., 2006; Ozaki et al., 2015)
0.1 M potassium phosphate buffer, 20% glycerol, 0.1 mM dithiothreitol, pH 7.4 (Wise et al., 1984)
0.1 M phosphate buffer, 1.15% KCl, pH 7.4 at 37°C (Taub et al., 2015)
50 mM Tris-HCl, 0.15 M KCl, 2 mM EDTA, pH 7.4 *

Homogeniser

Potter-Elvehjam Teflon type (Litterst et al., 1975; Aitio and Vainio, 1976; Nässberger et al., 1987; Pacifici et al., 1988; Tsoutsikos et al., 2004; Al-Jahdari et al., 2006; Ozaki et al., 2015) *
“Teflon pestle” (Jakobsson and Cinti, 1973; Jakobsson, 1974; Dai et al., 2004)
Teflon-glass (Okita et al., 1979; Prough et al., 1979)
Ultra Turrax (Tsoutsikos et al., 2004)
Dounce (Dohn and Anders, 1982)
Polytron (Wise et al., 1984) *
Waring Blender (Okita et al., 1979; Prough et al., 1979)
Not specified (Wistrand and Knuutila, 1989; Sharer et al., 1992; Lash et al., 1998; Cummings et al., 2001; Soars et al., 2001; Zordoky et al., 2011; Taub et al., 2015)

Microsome Isolation

Removal of cell debris/ mitochondria			Differential Centrifugation		
Centrifugal Force	Time	Reference	Centrifugal Force	Time	Reference
9000g	20 min	(Cummings et al., 2001; Al-Jahdari et al., 2006; Ozaki et al., 2015)	100000-105000g	30 min	(Aitio and Vainio, 1976)
9000g	15 min	(Pacifci et al., 1988)	100000-105000g	60 min	(Jakobsson and Cinti, 1973; Jakobsson, 1974; Litterst et al., 1975; Dohn and Anders, 1982; Nässberger et al., 1987; Pacifci et al., 1988; Sharer et al., 1992; Lash et al., 1998; Cummings et al., 2001; Soars et al., 2001; Tsoutsikos et al., 2004; Ozaki et al., 2015) *
12000g	10 min	(Jakobsson and Cinti, 1973; Aitio and Vainio, 1976)	100000-105000g	90 min	(Wistrand and Knuuttila, 1989; Sausen and Elfarra, 1990)
600g, 10000g	10 min (each)	(Tsoutsikos et al., 2004)	110,000g	70 min	(Dai et al., 2004)
10000g	15 min	(Wise et al., 1984; Soars et al., 2001)	78000g	60 min	(Okita et al., 1979; Prough et al., 1979)
5000g, 22000g	30 min, 15 min	(Okita et al., 1979; Prough et al., 1979)	100000g	Not specified	(Taub et al., 2015)
11000g	30 min	(Dai et al., 2004)	None	None	(Al-Jahdari et al., 2006)
1475g, 25000g	10 min, 10 min	(Wistrand and Knuuttila, 1989)	Not specified	Not Specified	(Zordoky et al., 2011)
500g, 10000g	5 min, 10 min	(Nässberger et al., 1987)	Pellet washing buffer		
9000g	90 min	(Litterst et al., 1975)	0.15 M KCl (Jakobsson and Cinti, 1973)		
48000g	30 min	(Sausen and Elfarra, 1990; Sharer et al., 1992)	1.15% KCl (Ozaki et al., 2015)		
10000g	20 min	(Jakobsson, 1974)	0.05 M potassium phosphate, 0.15 M KCl, 20% glycerol, pH 7.7 (Okita et al., 1979; Prough et al., 1979)		
15000g	5 min	(Lash et al., 1998)	10 mM potassium phosphate, 0.15 mM KCl, 1 mM EDTA, pH 7.4 (Dai et al., 2004)		
10000-12000g	20 min	*	10 mM Tris-HCl, 150mM KCl, 0.1 mM EDTA, pH 7.0 (Wistrand and Knuuttila, 1989)		
10800g	20 min	(Taub et al., 2015)			
Not Specified	Not Specified	(Zordoky et al., 2011)			

Calcium facilitated precipitation

0.25 M sucrose, 25 mM CaCl₂ added to 12000g supernatant, then centrifuged at 27000g for 30min. Pellet washed with 0.1 M Tris-HCl buffer pH 8.0 (Aitio and Vainio, 1976)

0.1 M Tris-HCl buffer, pH 8.0 (Aitio and Vainio, 1976)

0.15 M sucrose (Aitio and Vainio, 1976)

0.25 M sucrose, 10 mM TEA, 1 mM EDTA, pH 7.6 (Lash et al., 1998; Cummings et al., 2001)

0.25 M sucrose, 0.1 M potassium phosphate (pH 7.4) (Dohn and Anders, 1982)

0.1 M KH₂PO₄, 0.15 M KCl, 1.5 mM EDTA, pH 7.4 (Sharer et al., 1992)

0.15 M KCl, 10mM EDTA, pH 7.4 *

No pellet washing (Wise et al., 1984; Nässberger et al., 1987; Pacifici et al., 1988; Soars et al., 2001; Tsoutsikos et al., 2004; Al-Jahdari et al., 2006; Zordoky et al., 2011)

Storage

Buffer

0.25 M sucrose (Jakobsson and Cinti, 1973; Jakobsson, 1974; Litterst et al., 1975; Zordoky et al., 2011) *

0.1 M Na₂PO₄, 20% (w/v) glycerol, pH 7.4 (Tsoutsikos et al., 2004)

0.25 M sucrose, 5 mM HEPES, pH 7.4 (Soars et al., 2001)

0.05 M potassium phosphate, 0.1 mM EDTA, 0.02% NaN₃, 20% glycerol, pH 7.7 (Okita et al., 1979; Prough et al., 1979)

0.1 M Tris-HCl, 30% glycerol, pH 7.4 (Pacifici et al., 1988)

0.25 M sucrose and 1 mM EDTA, pH 7.4 (Dai et al., 2004)

0.15 M sucrose (Aitio and Vainio, 1976)

0.25 M sucrose, 10% (v/v) glycerol (Lash et al., 1998)

0.1 M KH₂PO₄, 0.15 M KCl, 1.5 mM EDTA, 20% (w/v) glycerol, pH 7.4 (Sharer et al., 1992)

0.25 M sucrose, 5 mM HEPES, pH 7.4 (Soars et al., 2001)

0.1 M potassium phosphate pH 7.4 (Wise et al., 1984)

20 mM Tris buffer, 0.25 M saccharose, 5.4 mM EDTA, pH 7.4 at 37°C (Taub et al., 2015)

Not Specified (Al-Jahdari et al., 2006)

Figure S1. A summary of different methods to isolate kidney microsomes from tissue collated from the literature.

Key: * - Method from a commercial source of human kidney microsomes

Figure S2

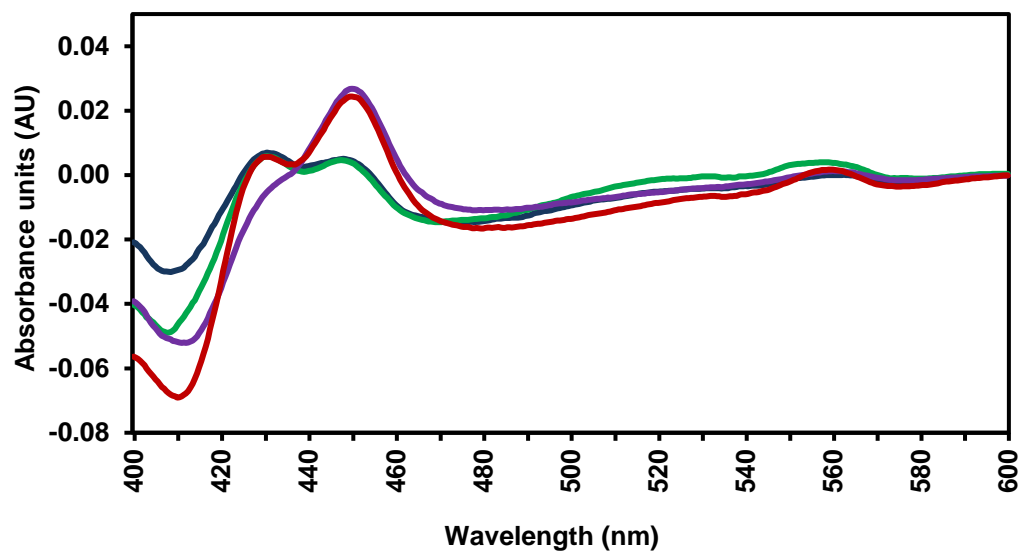


Figure S2. Representative UV/ Vis absorbance spectra from dithionite difference assay in dog kidney homogenate and microsomes. Buffer was modified to reduce cytochrome b_5 , cytochrome oxidase by inclusion of NADH and sodium succinate. Lines represent homogenate with normal (—) and modified buffer (—), and microsome with normal (—) and modified buffer (—). Data are the mean of duplicate measurements from a single experiment.

Figure S3

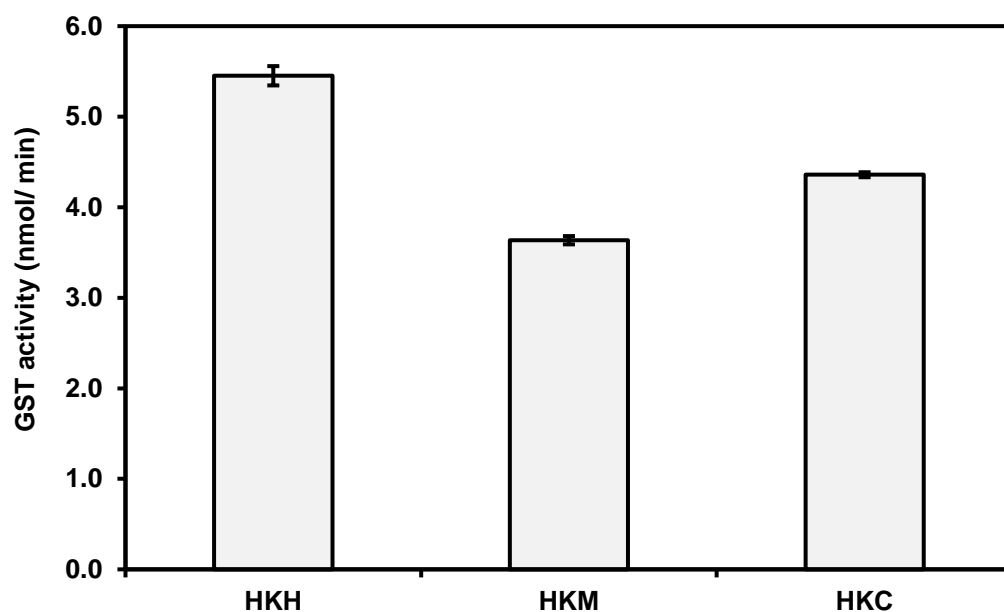


Figure S3. GST activity in human kidney homogenate, microsomes and cytosol from donor CMFT6. Protein concentrations were 50 $\mu\text{g}/\text{mL}$, which were higher than typically used (10 $\mu\text{g}/\text{mL}$) to ensure detection of potential GST activity in microsomal fraction. Mean and standard deviation (error bars) of data from three incubations in a single experiment are shown. HKH Human kidney homogenate; HKM Human kidney microsomes; HKC Human kidney cytosol

Table S1

Table S1 HPLC elution gradient for mycophenolic acid and warfarin (IS)

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	0	0	100
1	0	0	100
2	10	80	10
4	10	80	10
4.5	0	100	0
4.6	0	0	100
5.2	0	0	100

Solvent A: 0.05% formic acid in 90% water, 10% methanol; Solvent B: 0.05% formic acid in 10% water, 90% methanol; Solvent C: 1 mM ammonium acetate in 90% water, 10% methanol

Figure S4

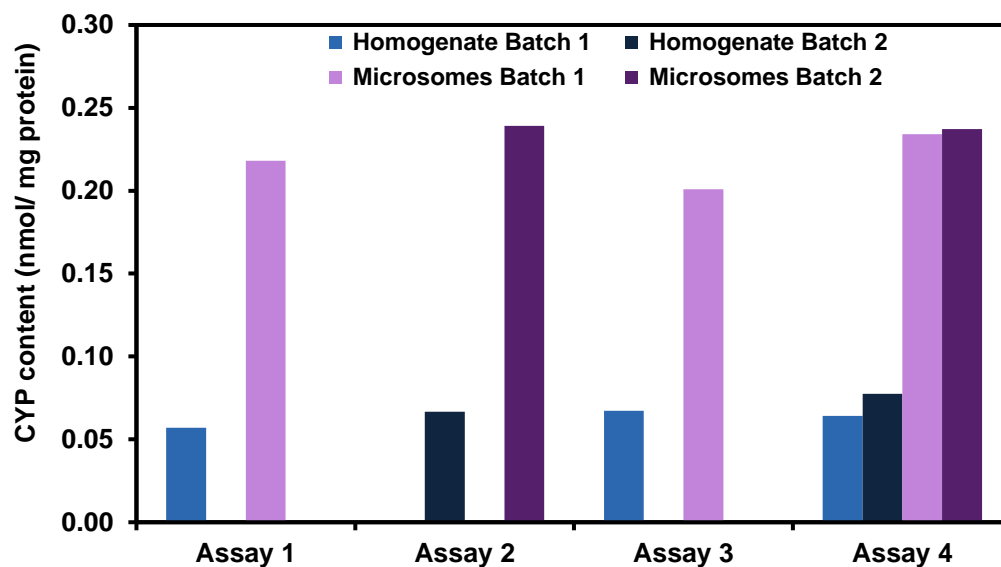


Figure S4. Inter-assay variability of CYP content measurements was similar to the inter-batch variability in paired homogenate and microsomes prepared from kidney tissue of a single dog.

Each bar represents the mean of two measurements from a single CYP content experiment in a single batch of homogenate or microsomes. For assay 4, measured CYP content varied by 13% and 1% between the two batches of homogenate and microsomes respectively.

Figure S5

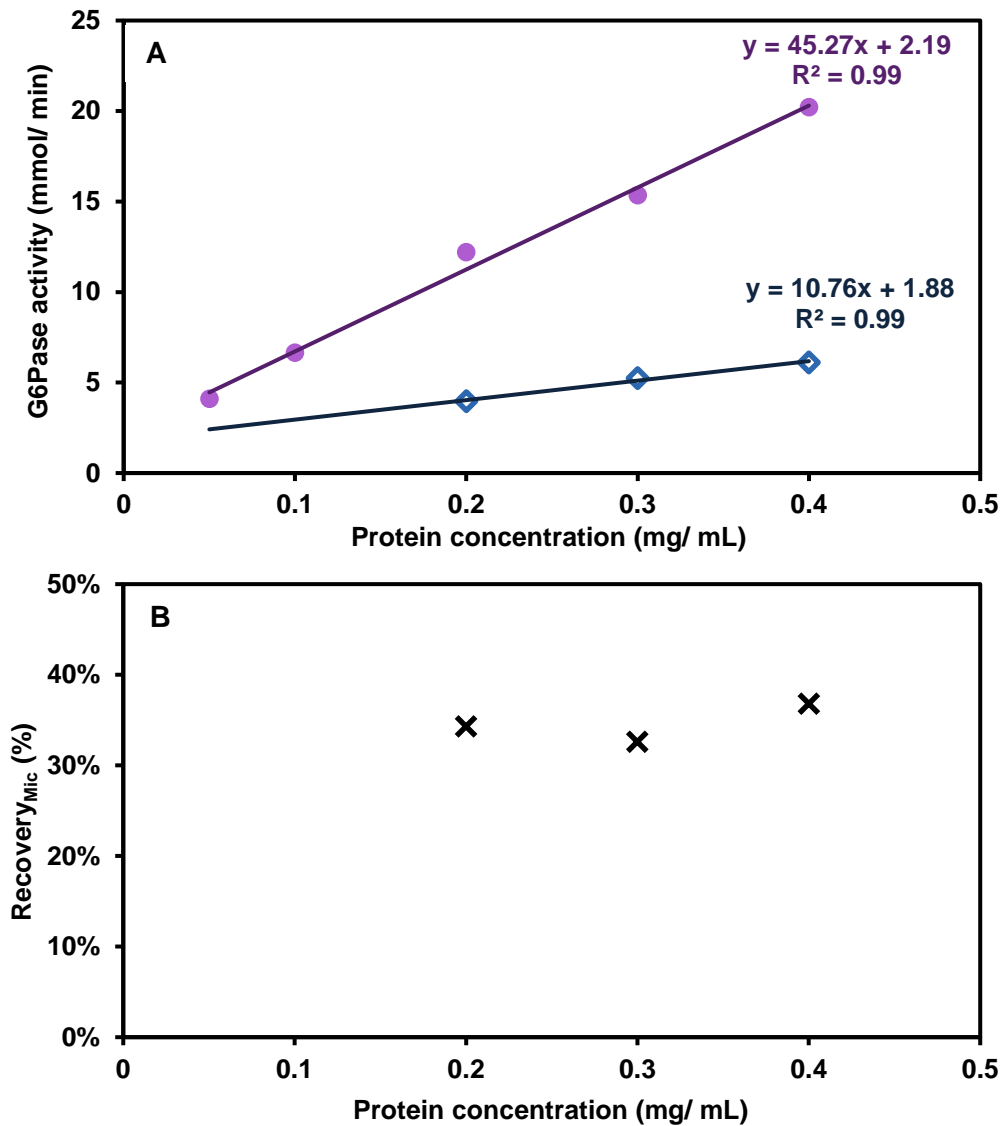


Figure S5 Assessment of linearity of G6Pase activity in dog kidney with respect to assay protein concentration (A) and impact on relationship between assay protein concentration and the estimated microsomal protein recovery (B).

Data are the mean of three incubations from a single experiment. In panel A, symbols indicate homogenate (◆) and microsomes (●) respectively, with linear lines of best fit and relevant equations shown.

Figure S6

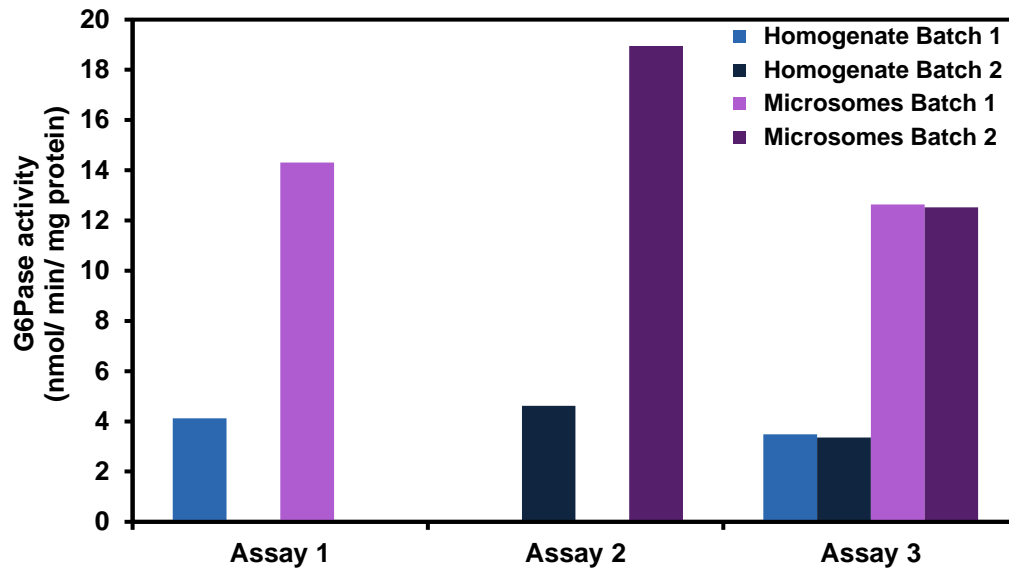


Figure S6. Inter-assay variability of G6Pase activity was greater than the inter-batch variability in paired homogenate and microsomes prepared from a single human kidney donor.

Each bar represents the mean of three incubations from a single G6Pase activity experiment in a single batch of homogenate or microsomes.

Figure S7

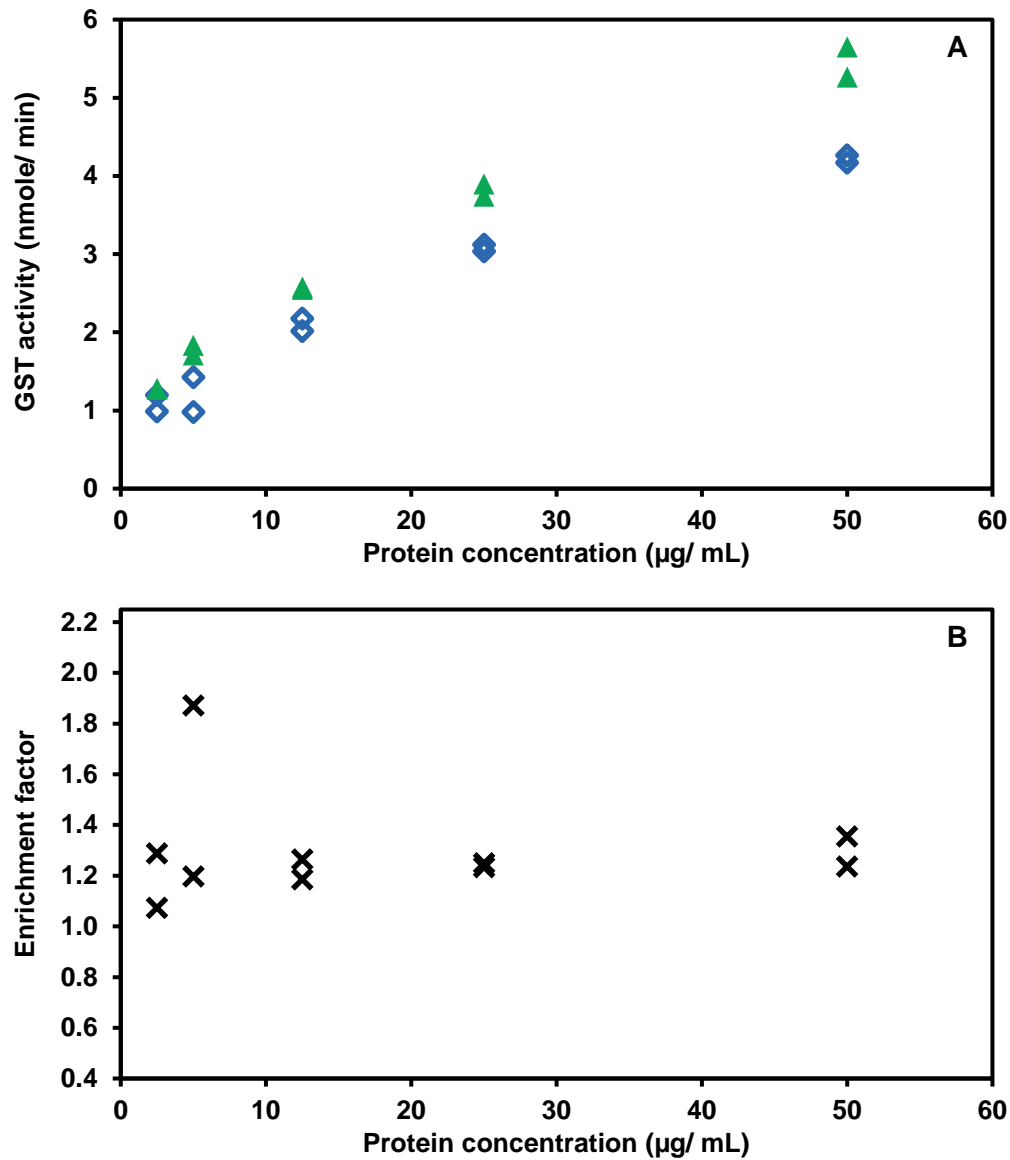


Figure S7 Assessment of GST activity linearity in rat kidney respect to assay protein concentration (A) and impact on relationship between assay protein concentration and the estimated cytosolic protein enrichment factor (B). Enrichment factor was calculated as the ratio of the cytosolic GST activity: homogenate GST activity. Each data point represents the mean of three incubations from a single experiment; data shown are from two separate experiments performed on the same day. In panel A, symbols indicate homogenate (◇) and cytosol (▲) respectively.

Table S2

Table S2 Microsomal protein marker data and MPPG estimates in kidney, liver and intestine for individual dogs.

General information					Fresh Tissue					
Dog ID #	Gender	Body Weight (kg)	Combined Kidney Weight (g)	Liver Weight (g)	Kidney			Intestine		
					Homogenate CYP content (nmol/ mg protein)	Microsomal CYP content (nmol/ mg protein)	MPPG _{K_{CYP}} (mg/ g kidney)	Intestine Region	Microsome CYP Content (nmol/mg protein)	MPPG _{I_{CYP}} (mg/ g intestine)
1	M	15.6	89.0	22.4	N/A	N/A	N/A	Prox 1	0.066	4.9
2	M	15.6	77.5	26.8	N/A	N/A	N/A	Prox 1	0.082	3.8
3	F	11.1	59.3	20.1	N/A	N/A	N/A	Prox 1	0.070	2.6
4	F	13.0	61.6	22.2	0.078	0.238	27.4	Prox 1	0.070	7.3
5	F	12.5	69.4	21.5	0.085	0.154	52.1	Prox 1	0.060	2.5
6	F	10.5	47.5	18.9	0.073	0.148	42.5	Prox 2	0.046	6.4
7	F	11.7	58.4	19.0	0.056	0.141	41.8	Prox 2	0.062	7.5
8	F	19.7	87.9	15.0	0.097	0.200	44.0	Prox 2	0.046	4.3
9	F	12.3	66.5	13.3	0.091	0.188	42.9	Prox 3	0.087	8.4
10	F	11.1	54.2	10.1	0.085	0.186	52.5	Prox 3	0.042	7.5
11	F	12.1	73.5	13.3	0.068	0.169	53.7	Prox 3	0.037	18.0
12	F	15.4	61.8	16.4	0.076	0.285	32.8	Distal	0.037	9.2
13	F	12.4	51.4	12.2	0.073	0.184	33.5	Distal	0.056	2.9
14	F	10.6	55.9	9.1	0.142	0.261	50.4	Distal	0.067	6.0
15	M	16.1	84.8	8.5	0.092	0.243	38.6	N/A	N/A	N/A
16	M	11.0	58.4	7.3	0.081	0.272	32.2	N/A	N/A	N/A
17	F	12.9	59.0	9.4	0.110	0.197	58.6	N/A	N/A	N/A
Average	4M	13.2	65.6	15.6	0.086	0.205	43.1		0.059	6.5
Standard Deviation	13F	2.5	12.8	5.8	0.021	0.047	9.5		0.016	4.0
CV		19%	19%	37%	24%	23%	22%		27%	61%
Range		10.5 - 19.7	47.5 – 89.0	7.3 - 26.8	0.056 - 0.142	0.141 - 0.285	27.4 - 58.6		0.037 - 0.087	2.5 - 18

	Frozen tissue					
	Kidney					
Dog ID #	Homogenate CYP content (nmol/ mg protein)	Microsomal CYP content (nmol/ mg protein)	MPPGK _{CYP} (mg/ g kidney)	Homogenate G6Psae activity (nmol/ min/ mg protein)	Microsome G6Psae activity (nmol/ min/ mg protein)	MPPGK _{G6Psae} (mg/ g kidney)
1	0.057	0.219	36.8	14.4	47.0	43.3
2	0.033	0.212	21.6	13.5	38.9	48.2
3	0.056	0.195	39.6	11.9	45.6	36.3
4	0.057	0.253	24.9	19.5	45.6	47.3
5	0.055	0.218	32.5	18.2	36.1	65.6
6	0.051	0.151	47.9	12.8	37.9	47.8
7	0.051	0.201	34.6	13.4	38.9	46.8
8	0.066	0.238	33.0	14.7	49.0	35.6
9	0.045	0.196	29.6	13.5	41.8	41.4
10	0.070	0.268	33.7	19.5	63.3	39.9
11	0.048	0.248	28.0	16.3	52.0	45.6
12	0.056	0.223	35.0	14.8	51.0	40.7
13	0.063	0.249	31.1	17.5	54.2	39.5
14	0.064	0.268	36.2	12.6	42.8	44.9
15	0.061	0.253	34.9	14.1	52.1	39.4
16	0.066	0.295	40.1	12.4	55.8	39.5
17	0.062	0.216	36.6	14.4	39.7	46.2
Average	0.056	0.230	33.9	14.9	46.6	44.0
Standard Deviation	0.009	0.035	6.1	2.4	7.5	6.9
CV	16%	15%	18%	16%	16%	16%
Range	0.033 - 0.070	0.151 - 0.295	21.6 - 47.9	11.9 - 19.5	36.1 - 63.3	35.6 - 65.6

Dog ID #	Frozen tissue					
	Liver					
	Homogenate CYP content (nmol/ mg protein)	Microsomal CYP content (nmol/ mg protein)	MPPGL _{CYP} (mg/ g kidney)	Homogenate G6Psae activity (nmol/ min/ mg protein)	Microsome G6Psae activity (nmol/ min/ mg protein)	MPPGL _{G6Pase} (mg/ g kidney)
1	0.153	1.023	33.9	16.2	81.7	44.9
2	0.128	0.711	41.7	18.7	81.4	53.4
3	0.102	0.626	38.9	18.2	80.1	54.0
4	0.083	0.619	32.6	14.5	68.4	51.2
5	0.106	0.651	39.8	17.6	74.8	57.8
6	0.077	0.630	43.6	12.6	63.3	70.8
7	0.130	0.700	39.3	14.9	43.0	73.6
8	0.096	0.343	53.8	17.4	54.1	61.8
9	0.117	0.767	42.3	18.7	62.1	83.8
10	0.096	0.567	40.9	20.5	83.9	59.0
11	0.132	0.656	42.5	15.5	56.8	57.9
12	0.125	0.690	41.6	18.8	76.3	56.8
13	0.145	0.774	44.0	16.7	53.7	73.0
14	0.121	0.658	43.7	22.7	67.3	80.4
15	0.087	0.538	32.1	19.1	67.4	56.3
16	0.112	0.715	42.8	22.2	83.0	73.4
17	0.113	0.645	44.6	19.1	66.1	73.8
Average	0.113	0.665	41.1	17.8	68.4	63.6
Standard Deviation	0.021	0.135	5.1	2.7	12.0	11.2
CV	19%	20%	12%	15%	18%	18%
Range	0.077 - 0.153	0.343 - 1.023	32.1 - 53.8	12.6 - 22.7	43.0 - 83.9	44.9 - 83.8

Figure S8

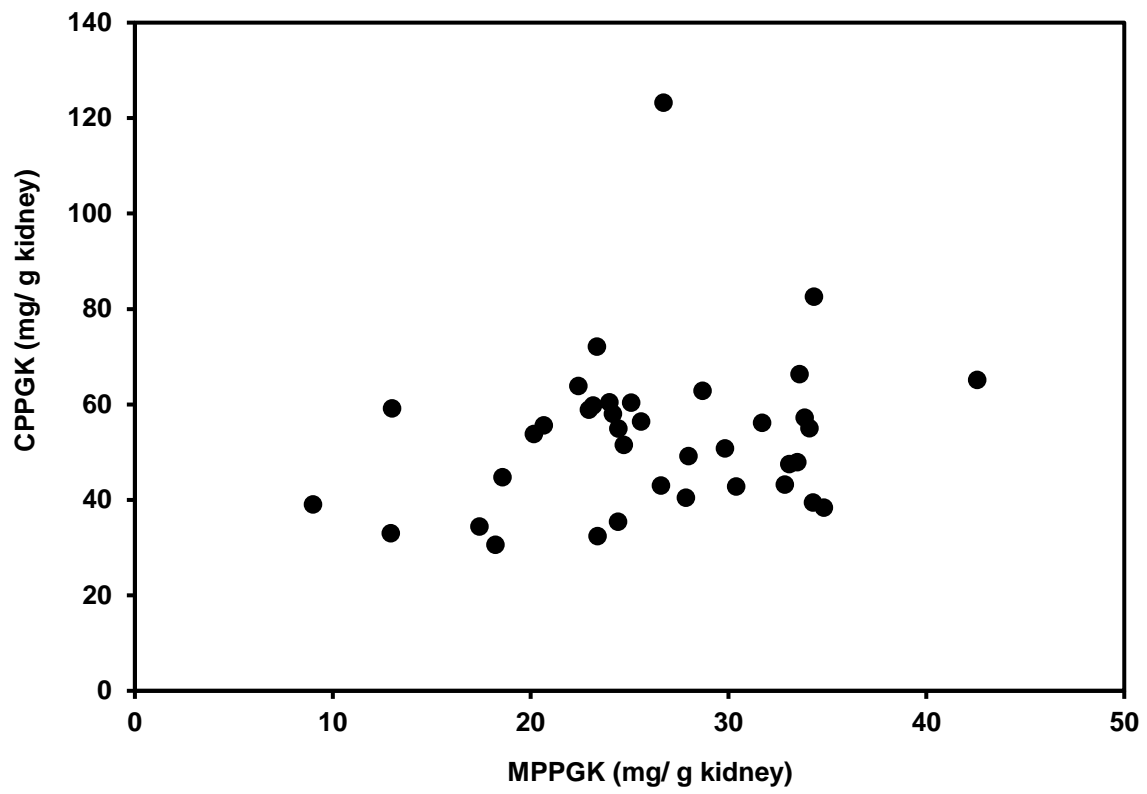


Figure S8. Comparison of MPPGK and CPPGK for human kidney microsomes from 38 donors

Figure S9

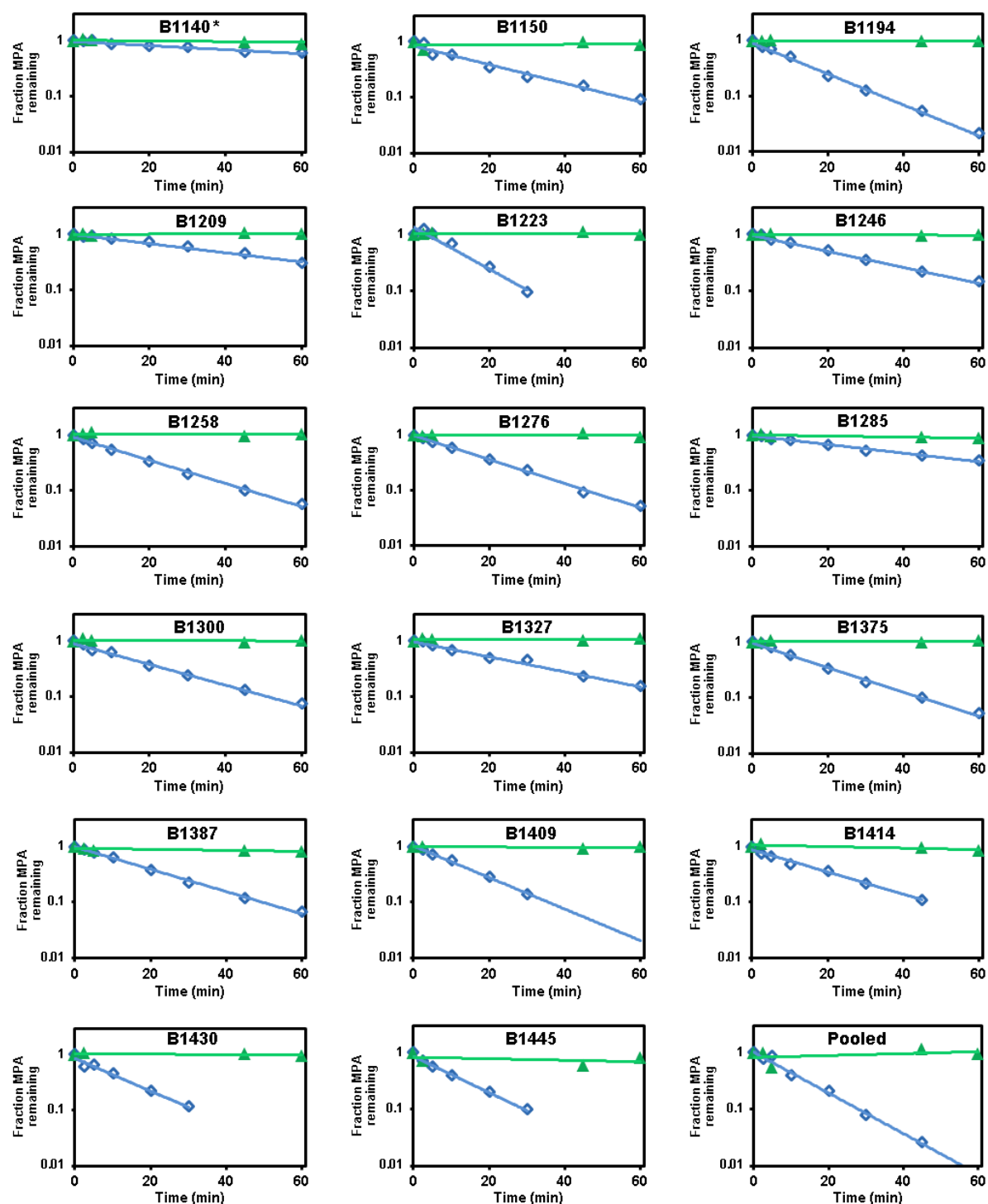


Figure S9 Fraction of mycophenolic acid (MPA) remaining over time in individual donor or pooled human kidney microsomes (0.25 mg/ mL) during glucuronidation substrate depletion assay.

◆ and ▲ represent incubations in the presence and absence of cofactor (UDPGA) respectively.

* Incubation conditions were modified for donor CMFT1 (protein concentration of 0.5 mg/ mL and 90 min incubation), as k could not be reliably quantified under standard conditions. Each point represents the mean of three measurements in a single experiment.

Figure S10

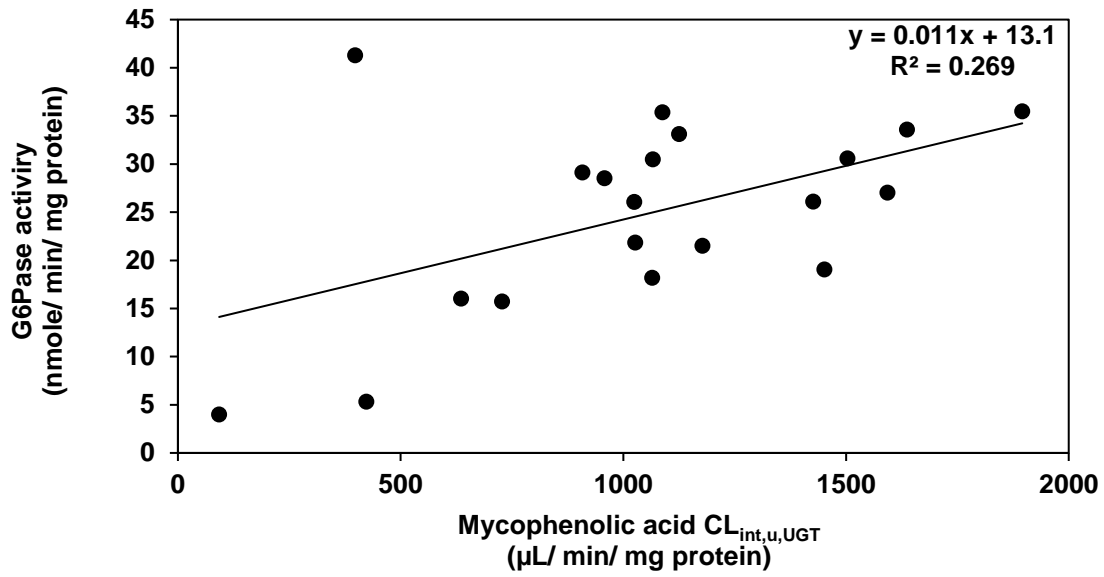


Figure S10 Comparison of G6Pase activity and mycophenolic acid CL_{int,u,UGT,HKM} for human kidney microsomes from 20 donors. Linear regression line and corresponding equation and R² are shown. Individual values are listed in Table S3

Table S3

Table S3 Demographics, protein recovery marker activities, subcellular protein content estimates, and mycophenolic acid *in vitro* glucuronidation data and IVIVE in individual human kidney samples.

Source	Donor ID	Demographics						
		Age (year)	Gender	Nationality/ Ethnicity	Weight (kg)	Height (m)	Smoking	Alcohol consumption (level/ units per week)
CMFT Biobank	CMFT1	66	Male	White British	72	1.72	No	Yes (20)
	CMFT2	77	Male	White British	NA	NA	No	No
	CMFT3	74	Female	White British	57.1	1.30	No	No
	CMFT4	65	Male	White British	127	1.88	Ex ^a	Yes (Socially)
	CMFT5	57	Female	British	78	1.81	Yes	Heavy in past
	CMFT6	78	Female	White British	51	1.56	No	NA
	CMFT7	47	Male	White British	76	1.75	Ex ^a	NA
	CMFT8	63	Male	White British	92	1.75	No	Yes (6)
	CMFT9	43	Male	NA	83.6	1.87	Yes (rarely)	Yes (4-6)
	CMFT10	45	Male	NA	108.6	1.72	No	Yes (35)
	CMFT11	76	Male	NA	83.5	1.74	NA	NA
	CMFT12	68	Male	NA	94.2	1.75	Ex ^a	Yes (Occasionally)
	CMFT13	73	Male	NA	94	1.85	Ex ^a	Yes (Occasionally)
	CMFT14	62	Male	NA	94	1.78	Yes (<10/day)	Yes (up to 28/ ex abusive)
	CMFT15	59	Male	NA	83	1.79	Ex ^a	No
	CMFT16	74	Male	NA	72.6	1.59	No	Yes (10)
	CMFT17	60	Male	NA	NA	NS	NS	NS
	CMFT18	83	Male	NA	92	1.7	Ex ^a	No
	CMFT19	77	Male	NA	82	1.74	No	Yes
	CMFT20	53	Female	NA	63	1.6	No	Yes (8)
Newcastle University	NC1	NA	NA	NA	NA	NA	NA	NA
	NC2	NA	NA	NA	NA	NA	NA	NA
	NC3	NA	NA	NA	NA	NA	NA	NA
	NC4	NA	NA	NA	NA	NA	NA	NA
	NC5	NA	NA	NA	NA	NA	NA	NA
	NC6	NA	NA	NA	NA	NA	NA	NA
	NC7	NA	NA	NA	NA	NA	NA	NA
	NC8	NA	NA	NA	NA	NA	NA	NA
	NC9	NA	NA	NA	NA	NA	NA	NA
	NC10	NA	NA	NA	NA	NA	NA	NA
	NC11	NA	NA	NA	NA	NA	NA	NA
	NC12	NA	NA	NA	NA	NA	NA	NA
	NC13	NA	NA	NA	NA	NA	NA	NA
	NC14	NA	NA	NA	NA	NA	NA	NA
	NC15	NA	NA	NA	NA	NA	NA	NA
	NC16	NA	NA	NA	NA	NA	NA	NA
	NC17	NA	NA	NA	NA	NA	NA	NA
	NC18	NA	NA	NA	NA	NA	NA	NA
n		20	4F/ 16M		18	18	2Y/ 9N/ 6Ex	11Y/ 4N
Average		65			84	1.72		
Standard Deviation		12			18	0.14		

CV Range		18% 43 – 83	22% 51 - 127	8% 1.30 - 1.88		
Source	Donor ID	Marker assays				
		Glucose-6-phosphatase activity (nmol/ min/ mg protein)		Glutathione-S-transferase activity (nmol/ min/ mg protein)		
		Homogenate	Microsomes	Homogenate	Microsomes	Cytosol
CMFT Biobank	CMFT1	1.7	4.0	115.0	65.2	180.2
	CMFT2	6.7	29.1	134.4	68.1	214.3
	CMFT3	7.2	26.1	180.3	136.5	320.9
	CMFT4	1.4	5.3	62.0	36.8	89.9
	CMFT5	9.8	35.5	173.9	124.7	240.4
	CMFT6	4.0	15.7	272.9	63.4	219.4
	CMFT7	9.4	30.5	131.0	77.3	143.9
	CMFT8	7.9	35.4	161.7	82.4	207.3
	CMFT9	11.4	41.3	353.2	235.9	415.7
	CMFT10	8.1	28.5	151.4	75.6	204.9
	CMFT11	6.1	16.0	242.8	119.1	332.2
	CMFT12	9.2	33.1	161.7	88.0	249.8
	CMFT13	7.2	21.9	88.6	42.2	161.2
	CMFT14	4.9	18.2	290.2	114.1	396.4
	CMFT15	4.3	21.5	220.0	102.0	374.1
	CMFT16	9.7	27.0	311.0	126.3	458.0
	CMFT17	6.0	19.1	207.8	120.1	408.2
	CMFT18	6.8	26.1	244.2	103.6	322.4
	CMFT19	7.4	30.6	185.6	82.9	258.2
	CMFT20	13.6	33.6	352.2	141.7	475.2
Newcastle University	NC1	8.8	30.3	320.8	133.8	339.6
	NC2	6.6	30.8	233.7	133.5	376.0
	NC3	12.9	39.4	350.3	150.3	521.9
	NC4	8.2	32.6	147.6	94.8	293.4
	NC5	3.0	17.2	173.3	71.3	200.1
	NC6	3.1	21.0	188.1	58.8	280.8
	NC7	8.6	38.4	216.8	120.1	431.9
	NC8	12.0	30.9	411.9	200.8	618.6
	NC9	8.8	24.9	188.5	75.8	313.7
	NC10	31.0	95.3	65.5	23.6	80.6
	NC11	7.3	22.9	153.5	123.5	252.6
	NC12	1.5	4.3	277.4	217.3	497.9
	NC13	6.9	22.2	236.4	120.8	447.1
	NC14	6.3	26.3	131.3	58.5	203.4
	NC15	8.7	26.4	190.7	81.0	293.0
	NC16	6.9	20.9	234.4	87.5	353.6
	NC17	14.1	48.1	372.5	134.3	443.5
	NC18	11.5	28.8	322.6	126.8	470.1
n		38	38	38	38	38
Average		8.1	27.9	217.3	105.7	318.2
Standard Deviation		5.0	14.8	87.8	46.1	125.8

CV Range		61% 1.4 – 31.0	53% 3.99 - 95.29	40% 62.0 - 411.9	44% 23.6 - 235.9	40% 80.6 - 618.6	
Source	Donor ID	Subcellular fractions (mg / g kidney)			Mycophenolic acid		
		MPPGK	CPPGK	S9PPGK	CL _{int,u,UGT,HKM} (µL/ min/ mg protein)	Scaled CL _{int,u,UGT,HKM} (mL/ min/ g kidney)	
						Scenario 1 ^b	Scenario 2 ^c
CMFT Biobank	CMFT1	34.8	38.4	73.2	93	1.0	3.2
	CMFT2	24.2	58.0	82.2	908	10.1	21.9
	CMFT3	26.6	43.0	69.6	1426	15.8	37.9
	CMFT4	28.7	62.9	91.6	423	4.7	12.1
	CMFT5	24.7	51.5	76.2	1896	21.0	46.8
	CMFT6	26.7	123.2	149.9	728	8.1	19.5
	CMFT7	34.3	82.6	116.9	1066	11.8	36.6
	CMFT8	23.4	72.1	95.5	1088	12.1	25.4
	CMFT9	24.0	60.5	84.4	398	4.4	9.5
	CMFT10	24.4	54.9	79.4	957	10.6	23.4
	CMFT11	33.8	57.2	91.1	636	7.1	21.5
	CMFT12	33.6	66.4	99.9	1125	12.5	37.8
	CMFT13	30.4	42.8	73.2	1026	11.4	31.2
	CMFT14	25.1	60.4	85.5	1065	11.8	26.7
	CMFT15	20.7	55.6	76.3	1178	13.1	24.3
	CMFT16	34.1	55.0	89.1	1593	17.7	54.3
	CMFT17	32.8	43.2	76.0	1451	16.1	47.7
	CMFT18	23.2	59.8	83.0	1024	11.4	23.7
	CMFT19	20.2	53.8	73.9	1503	16.7	30.3
	CMFT20	42.6	65.2	107.7	1637	18.2	69.7
Newcastle University	NC1	22.4	63.8	86.3	-	-	-
	NC2	12.9	33.0	45.9	-	-	-
	NC3	28.0	49.2	77.1	-	-	-
	NC4	18.2	30.6	48.8	-	-	-
	NC5	13.0	59.1	72.1	-	-	-
	NC6	9.0	39.0	48.1	-	-	-
	NC7	17.4	34.4	51.8	-	-	-
	NC8	23.4	32.4	55.8	-	-	-
	NC9	24.4	35.4	59.8	-	-	-
	NC10	25.6	56.5	82.0	-	-	-
	NC11	33.5	47.9	81.4	-	-	-
	NC12	34.3	39.5	73.7	-	-	-
	NC13	33.1	47.5	80.6	-	-	-
	NC14	18.6	44.7	63.3	-	-	-
	NC15	29.8	50.8	80.6	-	-	-
	NC16	31.7	56.1	87.8	-	-	-
	NC17	23.0	58.9	81.8	-	-	-
	NC18	27.8	40.5	68.3	-	-	-
n	38	38	38	20	20	20	
Average	26.2	53.3	79.5	1061.0	11.8	30.2	
Standard Deviation	7.1	16.6	19.4	454.5	5.0	15.9	
CV	27%	31%	24%	43%	43%	53%	

Range	9.0 – 42.6	30.6 - 123.2	45.9 - 149.9	92.6 - 1895.6	1.0 – 21.0	3.2 - 69.7
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Source	Donor ID	Mycophenolic acid			
		CL _{R,met,UGT} (mL/ min/ kg)		Predicted CL _{UGT} (mL/ min/ kg)	
		Scenario 1 ^b	Scenario 2 ^c	Scenario 1 ^b	Scenario 2 ^c
CMFT Biobank	CMFT1	0.08	0.16	2.94	3.03
	CMFT2	0.72	1.03	3.59	3.90
	CMFT3	1.11	1.69	3.97	4.55
	CMFT4	0.34	0.59	3.21	3.46
	CMFT5	1.44	2.03	4.30	4.89
	CMFT6	0.58	0.92	3.45	3.79
	CMFT7	0.84	1.64	3.71	4.50
	CMFT8	0.86	1.18	3.72	4.05
	CMFT9	0.32	0.47	3.19	3.33
	CMFT10	0.76	1.10	3.62	3.96
	CMFT11	0.51	1.02	3.38	3.88
	CMFT12	0.89	1.69	3.75	4.55
	CMFT13	0.81	1.42	3.68	4.29
	CMFT14	0.84	1.24	3.71	4.10
	CMFT15	0.93	1.14	3.79	4.00
	CMFT16	1.23	2.29	4.09	5.16
	CMFT17	1.13	2.06	3.99	4.92
	CMFT18	0.81	1.11	3.67	3.98
	CMFT19	1.16	1.39	4.03	4.25
	CMFT20	1.26	2.81	4.12	5.67
Newcastle University	NC1	-	-	-	-
	NC2	-	-	-	-
	NC3	-	-	-	-
	NC4	-	-	-	-
	NC5	-	-	-	-
	NC6	-	-	-	-
	NC7	-	-	-	-
	NC8	-	-	-	-
	NC9	-	-	-	-
	NC10	-	-	-	-
	NC11	-	-	-	-
	NC12	-	-	-	-
	NC13	-	-	-	-
	NC14	-	-	-	-
	NC15	-	-	-	-
	NC16	-	-	-	-
	NC17	-	-	-	-
	NC18	-	-	-	-
n	20	20	20	20	
Average	0.83	1.35	3.70	4.21	
Standard Deviation	0.34	0.63	0.34	0.63	
CV	41%	47%	9%	15%	
Range	0.08 – 1.44	1.16 – 2.81	2.94 - 4.30	3.03 – 5.67	

Source	Donor ID	Human kidney genotypes: Polymorphism [Gene; Position]						
		rs17863762 [UGT1A8; 830G>A]	rs17868320 [UGT1A9; - 2152C>T]	rs2741045 [UGT1A9; - 440T>C]	rs6714486 [UGT1A9; 275T>A]	rs72551330 [UGT1A9; 98T>C]	rs2741046 [UGT1A9; - 331C>T]	rs7438135 [UGT2B7; - 900G>A]
CMFT Biobank	CMFT1	GG	CC	CC	TT	TT	TT	AA
	CMFT2	GG	CC	CT	TT	TT	CT	GG
	CMFT3	GG	CC	CT	TT	TT	CT	GA
	CMFT4	GG	CC	TT	TT	TT	CC	GG
	CMFT5	GG	CC	CT	TA	TT	CT	AA
	CMFT6	GG	CC	CC	TT	TT	TT	GA
	CMFT7	GG	CC	CC	TT	TT	TT	GG
	CMFT8	GG	CC	CC	TT	TT	TT	GA
	CMFT9	AA	CC	CC	AA	TT	TT	GA
	CMFT10	GG	CC	CT	TT	TT	CT	GG
	CMFT11	GG	CC	CC	TT	TT	TT	GG
	CMFT12	GG	CC	CT	TT	TT	CT	GA
	CMFT13	GG	CC	CT	TT	TT	CT	GA
	CMFT14	GG	CC	CT	TT	TT	CT	AA
	CMFT15	GG	CC	CT	TT	TT	CT	AA
	CMFT16	GG	CC	CT	TT	TT	CT	AA
	CMFT17	GG	CC	CT	TT	TT	CT	GA
	CMFT18	GG	CC	CC	TT	TT	TT	GA
	CMFT19	GG	CC	CC	TT	TT	TT	AA
	CMFT20	GG	CC	CT	TT	TT	CT	GA
Newcastle University	NC1	-	-	-	-	-	-	-
	NC2	-	-	-	-	-	-	-
	NC3	-	-	-	-	-	-	-
	NC4	-	-	-	-	-	-	-
	NC5	-	-	-	-	-	-	-
	NC6	-	-	-	-	-	-	-
	NC7	-	-	-	-	-	-	-
	NC8	-	-	-	-	-	-	-
	NC9	-	-	-	-	-	-	-
	NC10	-	-	-	-	-	-	-
	NC11	-	-	-	-	-	-	-
	NC12	-	-	-	-	-	-	-
	NC13	-	-	-	-	-	-	-
	NC14	-	-	-	-	-	-	-
	NC15	-	-	-	-	-	-	-
	NC16	-	-	-	-	-	-	-
	NC17	-	-	-	-	-	-	-
	NC18	-	-	-	-	-	-	-
n		1A/ 19G	20C	1T/ 11CT/ 8C	18T/ 1TA/ 1A	20T	1C/ 11CT/ 8T	5G/ 9GA/ 6A

^a Stopped smoking > 5 years before surgery; ^b MPPGK = 11.1 mg/ g kidney used for scaling; ^c MPPGK values obtained for individual donors used for scaling; NA Demographics data not available; - No data generated.

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