Supplemental Data File

ESI-MSMS analyses: ESI-MS/MS was performed on a Waters Quattro Micro instrument operating in the positive-ion mode.

Data acquisition was carried out with MassLynx 4.1 software with the following settings: capillary voltage, 3500 V; cone voltage, 80 V; extractor, 2 V; RF, 0.0 V; source temperature, 80 °C; desolvation temperature, 250 °C; cone gas flow, 30 L/h; desolvation gas flow, 550 L/h; collision gas flow, 0.20 mL/min; LM 1 resolution, 15; HM 1 resolution, 15; ion energy 1, 0.2; MS/MS mode entrance, 15; MSMS collision energy, 30 eV (Gal-6S-P) and 30 eV (Gal-6S-IS); MS/MS mode exit, 15; LM 2 resolution, 15.0; HM 2 resolution, 15.0; ion energy 2, 2.0; Multiplier, 650; collision cell pressure, $< 10^{-4}$ mbar; collision gas, argon. Multiple-reaction-monitoring mode was used for *m/z* 589.2 $\rightarrow$ 489.1 and 603.2 $\rightarrow$ 503.1 transitions with the following settings: dwell time, 0.1 s; delay, 0.02 s.

The sample (10 µL of the 30 µL sample in 80/20 acetonitrile/ water with 0.2% formic acid, see main text) was injected into the mass spectrometer with a flow-rate of 0.1 mL/min. Data was collected during 1 minute of infusion, and after 1 min the MS/MS signal has returned to the background level.

The amount of product was calculated from the ion abundance ratio of product to internal standard, minus that from a minus DBS blank control, multiplied by the amount of added internal standard. Enzymatic activity was calculated from the amount of

product divided by the incubation time and the volume of blood (1.6 µL of blood in a 2-mm DBS punch).

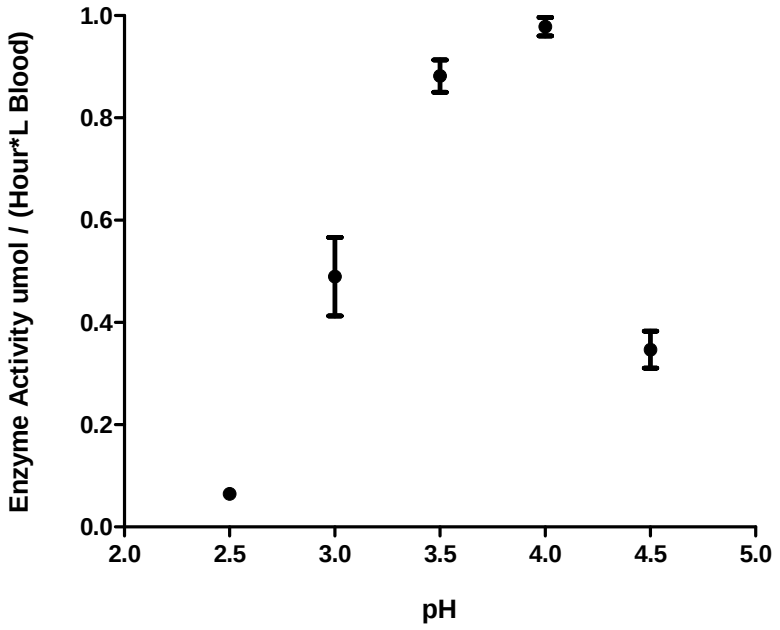
Supplementary Data Table 1. GALNS activities for individual DBS (ethyl acetate extraction workup)

GALNS activity (µmol/h/L blood)			
No Substrate control[1]	Minus DBS Blank control	MPS IV A patients	Healthy newborns
0.00027	0.0032	0.013	0.317
0.00039	0.0036	0.0062	0.395
0.0005	0.0035	0.0019	0.462
0.00093		0.0044	0.187
0.00046		0.014	0.109
0.00058		0.0166	0.149
0.00082		0.0173	0.367
0.00055		0.0123	0.187

0.00429	0.0188	0.306
0.00259		0.274
0.00393		0.224
0.00135		0.318
0.00407		0.233
0.0008		0.203
0.00427		0.322
0.00322		0.274
0.00132		0.292
0.00073		0.121
0.00265		0.289
0.0008		0.55
0.00088		0.265
0.00404		0.237
0.00259		0.42
0.00067		0.196
0.00317		0.259
0.00467		0.541
0.00174		0.143
0.00139		0.185
0.00352		0.294
0.00205		0.256

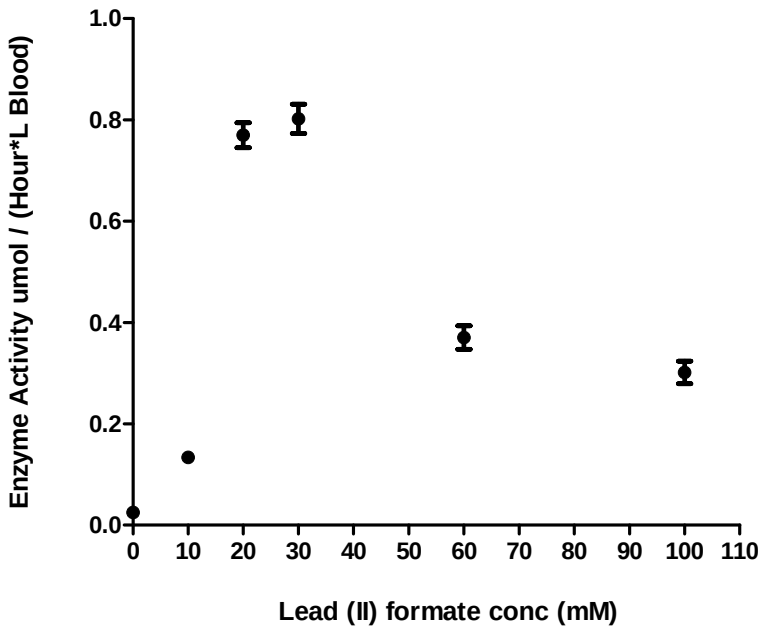
[1]These assays were carried out with 30 different healthy newborn DBS, each containing all assay components except GALNS-S.

Supplementary Data Figure 1



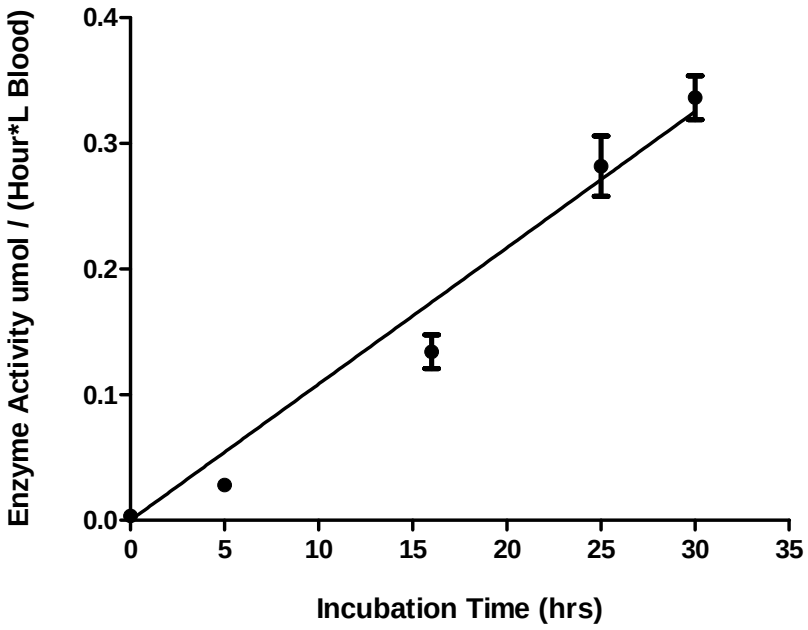
Amount of GALNS generated product measured in DBS as a function of the pH of the enzymatic reaction during incubation. Reactions were carried out at 37 °C for 16 h using the standard assay with solid-phase extraction given in the main text. Error bars are shown for triplicate analyses.

Supplementary Data Figure 2



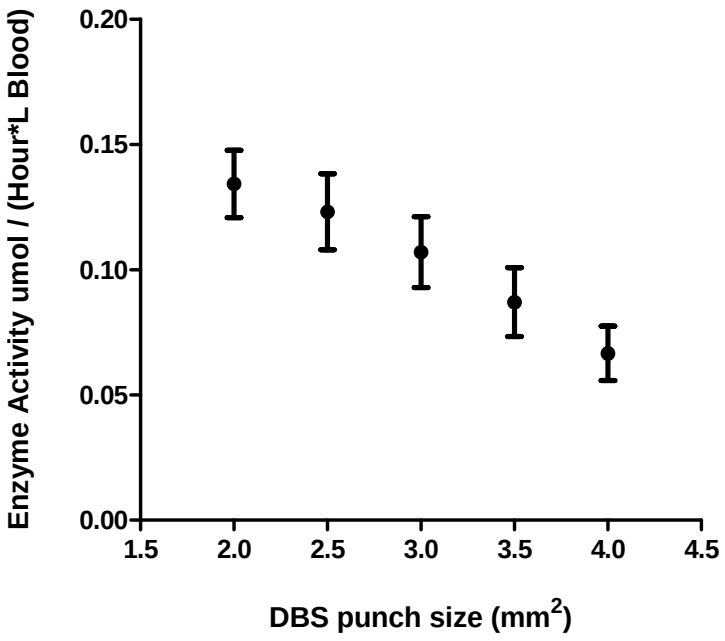
Amount of GALNS generated product measured in DBS as a function of the concentration of lead(II) formate. Reactions were carried out at 37 °C for 16 h using the standard assay with solid-phase extraction given in the main text. Error bars are shown for triplicate analyses.

Supplementary Data Figure 3



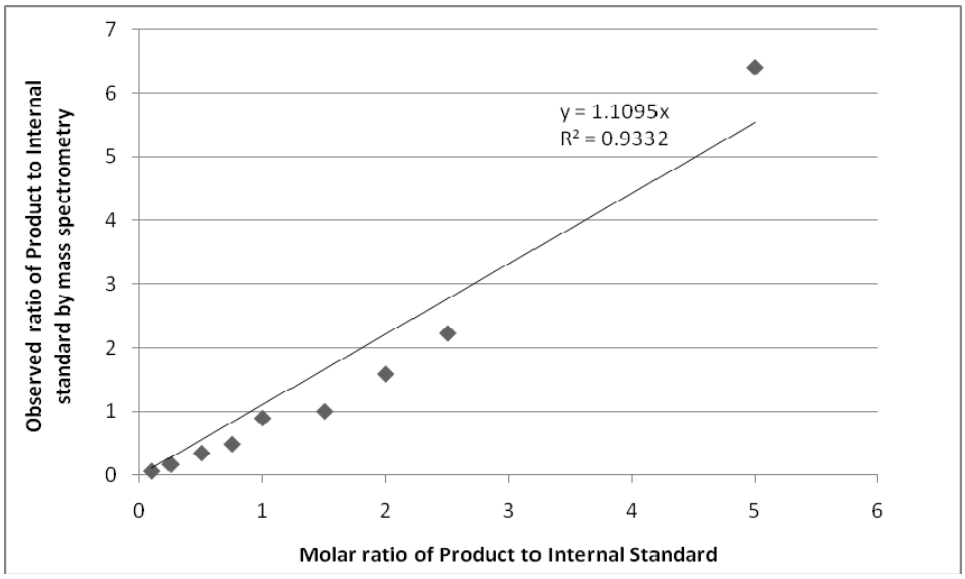
Amount of GALNS generated product measured in DBS as a function of the incubation time. Reactions were carried out at 37 °C using 0.2 mmol/L GALNS-S and 0.1 nmol GALNS-IS using the conditions given in the main text (solid-phase extraction method). Error bars are shown for triplicate analyses. The solid line shows the linear regression fit of the data.

Supplementary Data Figure 4



Amount of GALNS generated product measured in DBS as a function of the size of the DBS punch. Reactions were carried out at 37 °C for 16 h using 0.2 mmol/L GALNS-S and 0.1 nmol GALNS-IS using the conditions given in the main text (solid phase extraction method). Error bars are shown for triplicate analyses.

Supplementary Data Figure 5

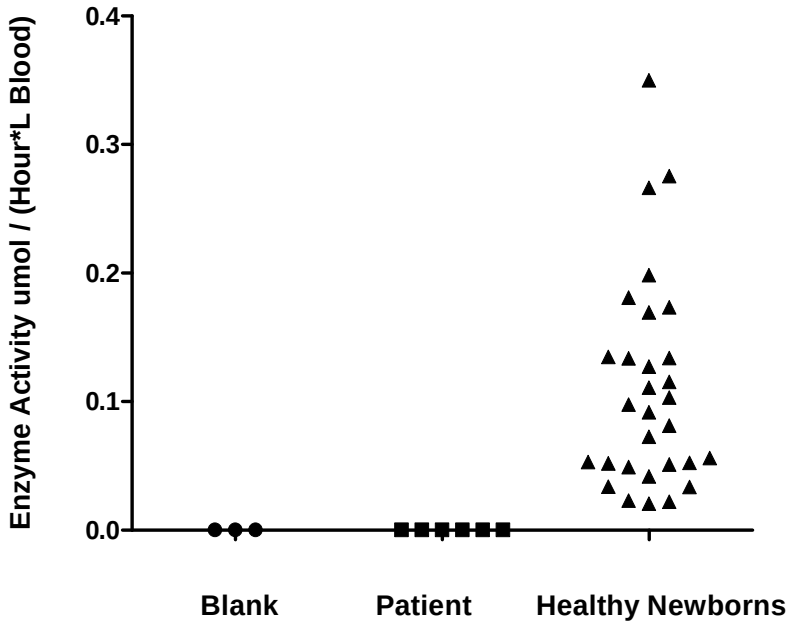


GALNS-P/GALNS-IS ion ratio observed by ESI-MSMS as a function of the relative amount of GALNS-P and GALNS-IS added to the assay. Samples contained all assay components except substrate, a fixed amount of GALNS-IS (0.1 nmol) and various amounts of GALNS-P. The solid line shows the linear regression fit of the data.

Supplementary Data Table 2. GALNS activities for individual DBS with solid-phase extraction using 0.2 mM substrate

Minus DBS Blank control	MPS IV A patients	Healthy newborns
0.00039	0.00039	0.023
0.00028	0.00039	0.115
0.00025	0.00043	0.103
	0.00043	0.127
	0.00042	0.022
	0.00041	0.35
		0.051
		0.041
		0.133
		0.091
		0.266
		0.275
		0.052
		0.173
		0.180
		0.133
		0.097
		0.056
		0.081
		0.110
		0.198
		0.020
		0.049
		0.169
		0.033
		0.053
		0.134
		0.033
		0.072
		0.051

Supplementary Data Figure 6. GALNS activities for individual DBS with solid-phase extraction using 0.2 mM substrate



Supplementary Data Table 3. GALNS activities for individual DBS with solid-phase extraction using 2 mM substrate

Minus DBS Blank control	MPS IV A patients	Healthy newborns
0.0037	0.023	1.66
0.0035	0.026	3.54
0.0027	0.012	1.35
	0.022	1.01
	0.006	2.39
	0.0007	1.36
	0.023	2.01
	0.010	1.37
	0.014	2.85
		2.31
		2.27
		1.33
		3.13
		4.37
		1.72
		1.09
		2.98
		1.34
		0.64
		1.79
		1.85
		2.43
		1.55
		2.35
		2.29
		1.87
		1.98
		1.69
		1.62
		1.83

Supplementary Data Figure 7. GALNS activities for individual DBS with solid-phase extraction using 2 mM substrate