Supporting information

Evaluation of the Performance and Hematocrit Independence of the HemaPEN as a Volumetric Dried Blood Spot Collection Device

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Supplementary data Table S-1

Results analyte and IS-compensated matrix effect

Table S-1. Analyte and IS-compensated matrix effect for caffeine and paraxanthine at two different concentration levels (n= 9).

	Caffeine		Paraxanthine	
	Low QC	**High QC**	**Low QC**	**High QC**
	Analyte matrix effect			
Mean of 9 donors (%)	97%	94%	105%	103%
(%CV)	6.95%	3.62%	5.15%	2.29%
	IS compensated matrix effect			
Mean of 9 donors (%)	104%	99%	101%	100%
(%CV)	4.78%	4.24%	4.96%	5.40%

Supplementary data Table S-2

Results stability study hemaPEN®

Table S-2. Stability data for caffeine and paraxanthine in hemaPEN® after storage for 4 days at 60°C and 2 months at room temperature, kept in their original package. Expressed in % difference with the nominal value.

	4 days at 60°C (% difference) (n = 3)			2 months at T_r (% difference) (n =3)	
QC	**Caffeine**	**Paraxanthine**	Concentration (µg/mL)	**Caffeine**	**Paraxanthine**
Low QC	-0.56%	2.50%	0.12 or 0.06	-3.33%	-10.22%
High QC	-1.92%	0.92%	8.0 or 4.0	-11.18%	-8.08%

Supplementary data Figure S-1

Bland-Altman comparison for whole liquid blood to 3 mm partial-punch DBS

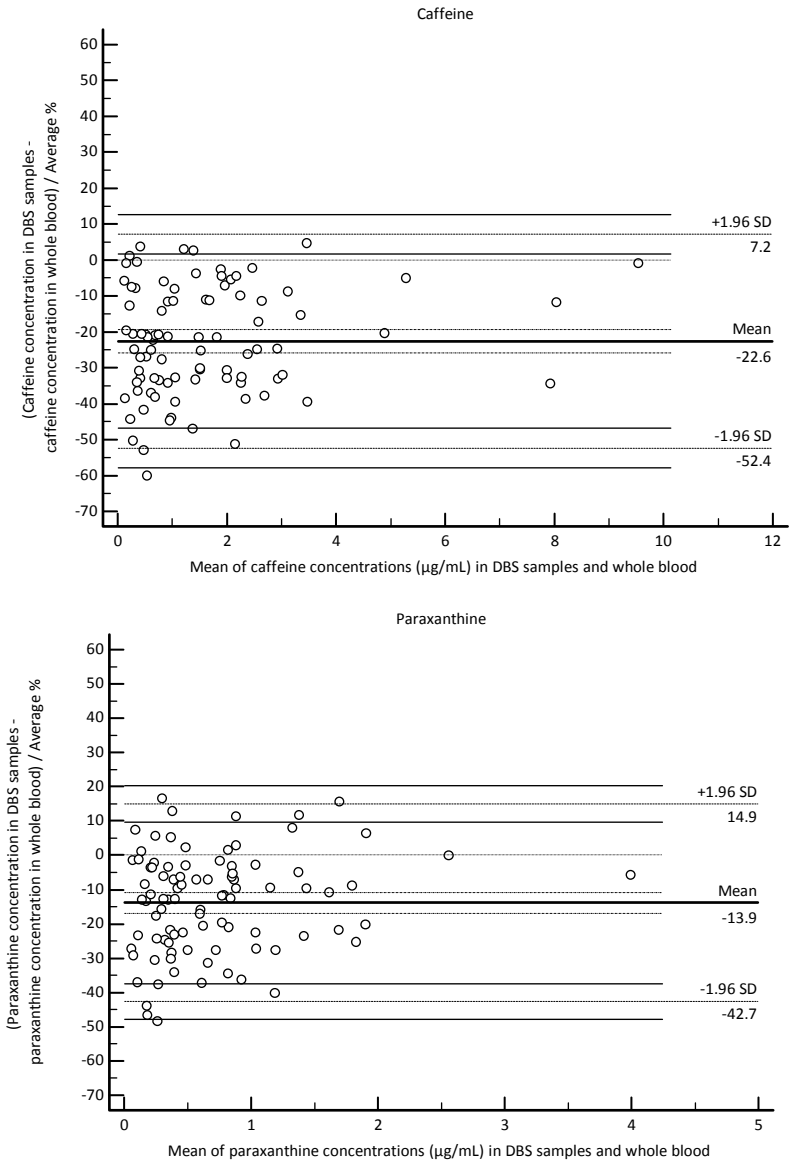

Figure S-1. Bland–Altman plots for the comparison between whole blood and 3 mm partial-punch DBS concentrations for caffeine (n=88) and paraxanthine (n=91). Mean differences and limits of agreement (LoA) are represented by full lines, 95% confidence limits by broken lines.

Supplementary data Figure S-2

Regression analyses of the individual hemaPEN®-whole blood differences as a function of hct.

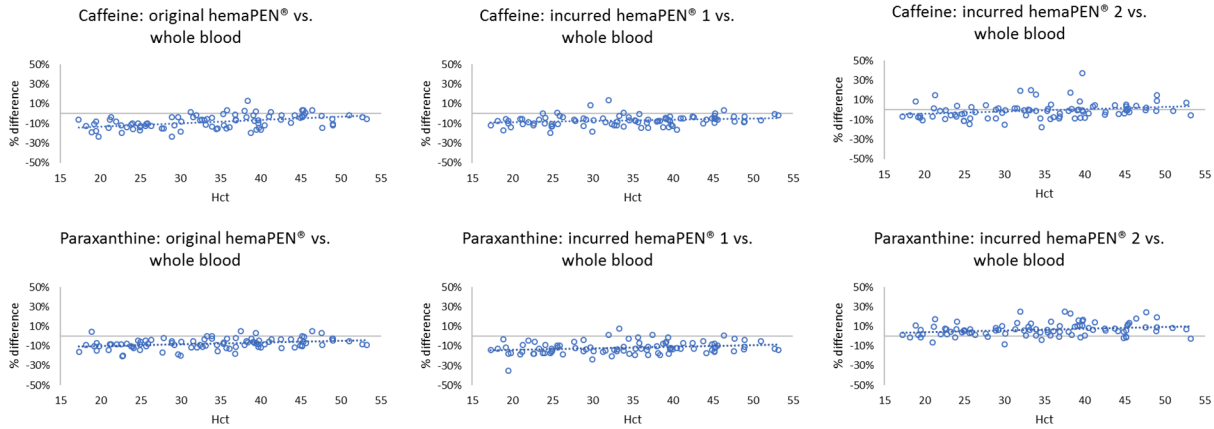

Figure S-2. % difference between hemaPEN® DBS and whole blood concentrations, plotted against hct for caffeine and paraxanthine. Broken lines represent linear regression lines. The respective slopes for original hemaPEN® DBS, incurred hemaPEN® DBS 1 and incurred hemaPEN® DBS 2 were 0.003388 (95%CI [0.00198;0.00480]), 0.001301 (95%CI [0.00012;0.00248]) and 0.002309 (95%CI [0.00045;0.00417]) for caffeine and 0.001898 (95%CI [0.000768;0.003028]), 0.001587 (95%CI [0.000238;0.002935]) and 0.001769 (95%CI [0.00032;0.003219]) for paraxanthine. The respective intercepts for original hemaPEN® DBS, incurred hemaPEN® DBS 1 and incurred hemaPEN® DBS 2 were -0.1966 (95%CI [-0.2466;-0.1466]), -0.1146 (95%CI [-0.1565;-0.0728]) and -0.0860 (95%CI [-0.1520;-0.0200]) for caffeine and -0.1408 (95%CI [-0.1809;-0.1008]), -0.1722 (95%CI [-0.2200;-0.1244]) and 0.00756 (95%CI [-0.0438;0.0590]) for paraxanthine.

Supplementary data Figure S-3

Box-and-Whisker plots evaluation hemaPEN® robustness to sample volume, device lot, analytical operator and sample stability.

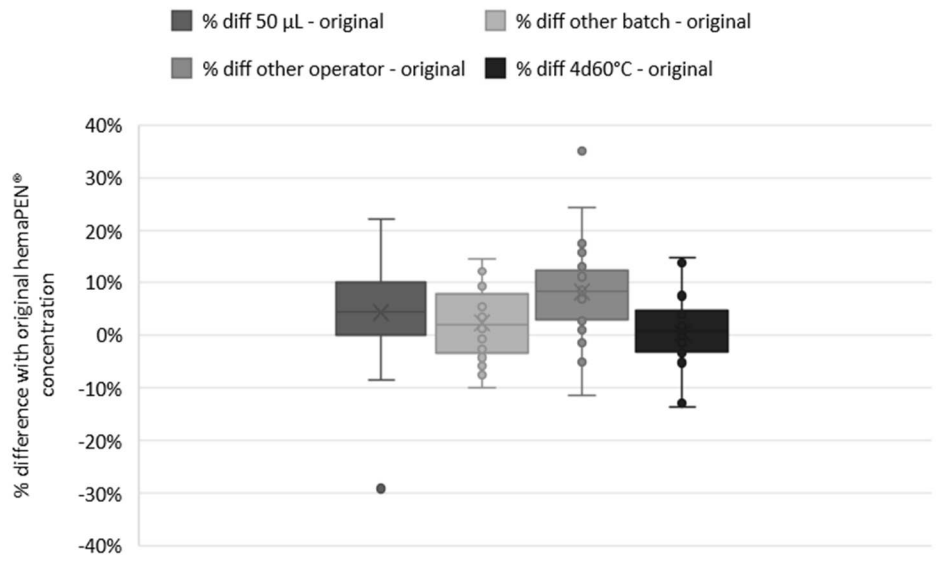

Figure S-3A. 50 µL, other batch, other operator and the set kept for 4 days at 60°C compared to the original hemaPEN® measurement for caffeine in % difference. Based on a box-and-whisker plot two outliers can be identified.

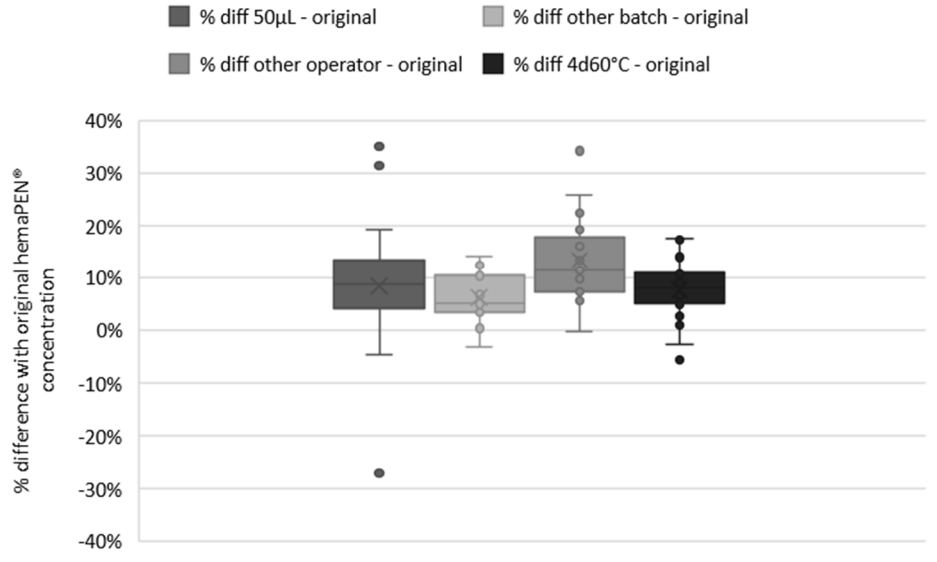

Figure S-3B. 50 µL, other batch, operator and the set kept for 4 days at 60°C compared to the original hemaPEN® measurement for paraxanthine in % difference. Based on a box-and-whisker plot five outliers can be identified.