Performance Evaluation of Commercially Available Kits for the Measurement of Human Hepcidin

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Introduction: The discovery of the iron-regulatory hormone hepcidin has revolutionized our understanding of iron biology and the associated disorders that arise from disruptions in iron homeostasis. The measurement of serum hepcidin should advance diagnosis and treatment of these conditions but a gold standard is still lacking, and efforts toward harmonization are ongoing. Meanwhile, an internet search yields over 150 human hepcidin kits from more than 20 suppliers.

Objective: The goal of this study was to assist researchers in selecting the optimal kit for their experimental needs and provide conversion equations to normalize hepcidin values between kits. We selected the top 5 kits based on usage cited in the literature: Intrinsic LifeSciences (ICE-007), BMA Biomedicals (S-1337), R&D Systems (DHP250), DRG International (EIA5782R), and Biomatik (EKU08553). These kits were evaluated for accuracy, precision, linearity, and matrix interference. The kits were then assessed for diagnostic accuracy in predicting iron deficiency (ID).

Methods: The performance evaluation was carried out according to CLIA guidelines (Westgard, 2008) by a third party CRO. Accuracy was assessed with spiked serum samples (n = 24) across the physiological range (0-170 ng/ml). The concentration of synthetic hepcidin peptide used for spiking was quantitated independently by spectrophotometry: mg/ml = (A215-A225) x 0.144 (Manchester, 1996). Coefficients of variation (CV) were calculated from replicate (n = 4) measurements across multiple kits (n = 4) using high, medium, and low hepcidin samples. Linearity was assessed using a high hepcidin sample serially diluted 1:2, 1:4, 1:8, and 1:16. Matrix interference was evaluated using matched serum, Li heparin plasma, and EDTA plasma samples (n = 5). The ability to predict ID (Ft < 30 ng/ml) in non-anemic (Hb > 12.5 g/dL) first-time blood donors was evaluated by ROC analysis (n = 35 ID, n = 35 iron replete). The optimal hepcidin cutoff was selected from the maximum Youden index [(sensitivity + specificity)-100]/100.

Results: The performance evaluation results are in Table 1. Each of the kits measured the spiked samples at different levels but with a high degree of correlation. The exception for this was the Biomatik assay which did not detect hepcidin in any of the samples and therefore was excluded from further analysis. The remaining kits had Pearson R values > 0.96 and p values < 0.001. However, the slopes of the regressions ranged from 1.07 to 0.20 (Figure 1). The clear differences in absolute values measured by each kit demonstrate the need for conversion equations to accurately compare datasets generated from the different kits (Table 2). The

average intra-assay precision across high, medium, and low hepcidin samples ranged from 6.4% to 12.7% CV while the inter-assay precision spanned a much greater range from 7.3% to 40.2%. The average linearity across all dilutions ranged from -20.0% to 5.9% relative error (RE). The level of agreement across 3 matched sample matrices was measured by CV and ranged from 6.4% to 21.3%.

The kits were then assessed for their ability to predict ID in non-anemic first-time blood donors. The correlation of hepcidin to ferritin in a log-log plot was strong for all 4 kits ranging from 0.699 to 0.867 (Figure 2). ROC analysis reveals relatively similar AUCs for each of the kits (0.86-0.9) demonstrating comparable ability to predict ID (Figure 3). Similarly, the percent of correctly classified donors were similar (79%-81%).

Conclusion: Several commercial ELISA kits adequately measure relative concentrations of serum hepcidin but with varying degrees of precision, particularly inter-assay. While there is a clear need for calibrator harmonization, our conversion equations can aid in the comparison of raw values between kits. The ability to predict ID in non-anemic donors is very comparable between tested kits with highly overlapping 95%CI for ROC AUC and % correctly classified. These characteristics can contribute to the scientific rigor of hepcidin biology research.

Kit Method	Spike Recovery[a]		Average Precision, %CV (range)		Linearity, %RE (range, n=2)[b]	Matrix interference, %CV[c]
	Slope (95%CI)	R	Intra-assay (n=4)	Inter-assay (n=3)		
A	1.07 (1.00-1.13)	0.991	9.1 (7.6-9.8)	7.3 (5.6-9.0)	5.9 (2.9 to 9.1)	8.2
B	0.58 (0.51-0.65)	0.962	12.7 (7.4-18.7)	40.2 (26.2-57.5)	-20.0 (-26.8 to -13.8)	21.3
C	0.68 (0.63-0.73)	0.987	6.4 (4.1-10.1)	26.7 (22.5-33.8)	-7.6 (-2.7 to -11.0)	9.3
D	0.20 (0.19-0.22)	0.984	7.5 (3.5-10.4)	17.0 (9.1-21.2)	-0.7 (-9.6 to 0.6)	6.4

Table 1. Performance characteristics of commercially available human hepcidin kits. A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R).
[a]Serum spiked with synthetic hepcidin (n = 24, 0-170 ng/ml)
[b]Serum spiked with a high level of hepcidin and serially diluted 1:2, 1:4, 1:8, and 1:16
[c] Matched serum, Li heparin plasma, and EDTA plasma (n = 5)

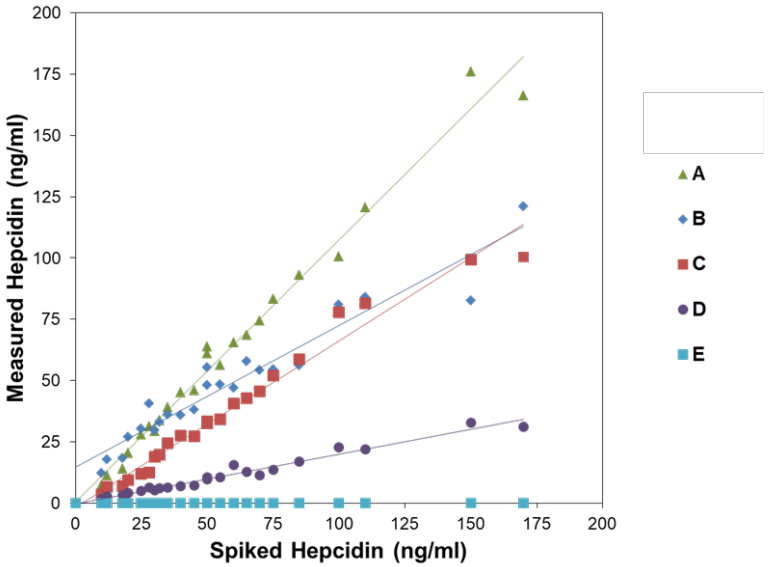

Figure 1. Hepcidin kit spike recovery. A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R); E, Biomatik (EKU08553).

		X =		
		A	B	C
Y =	B	2.8643(X)-33.307 R = 0.972	0.4051(X)-1.7186 R = 0.940	0.4219(X)-3.9054 R = 0.958
	C		0.1366(X)+3.4145 R = 0.912	0.1379(X)+1.4131 R = 0.945
	D			0.9002(X)-0.4261 R = 0.882

Table 2. Conversion equations between different hepcidin kits. A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R). Pearson correlation equations and coefficients for serum samples (n =70) measured by each kit. Plugging in the hepcidin value (ng/ml) measured by the kit in each column (X) into the respective equation converts that value to the corresponding kit (Y).

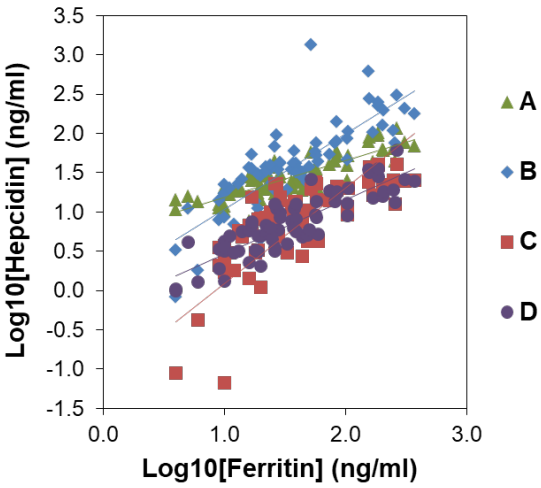

Figure 2. Correlation of serum ferritin and hepcidin. A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R). The Pearson correlation coefficients are 0.856, 0.816, 0.699, 0.867, respectively.

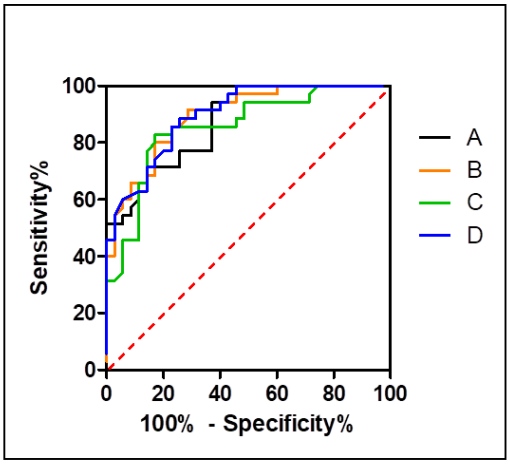

Figure 3. ROC curve for prediction of ID. A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R). The AUCs are 0.87, 0.89, 0.86, 0.90, respectively.