

# Researcher's Guide to Hepcidin Testing

Selecting the Optimal  
Hepcidin Test Kit



# Method Comparison

## Study Goal

Quantify the performance characteristics of available hepcidin kits to assist researchers in making informed kit selection decisions related to hepcidin research.

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# Introduction



## Why test for hepcidin?

### Iron Disorders

Iron metabolism disorders are often accompanied by alterations in hepcidin levels.

### Hepcidin is an important biomarker, which can:

- predict iron deficiency (ID)<sup>1-4</sup>
- distinguish iron deficiency anemia (IDA) from anemia of chronic disease (ACD)<sup>5</sup>
- guide iron therapy treatments<sup>6,7</sup>

### Therapeutics<sup>8-10</sup>

Hepcidin is the therapeutic target for:

- **Iron overload (IO) disorders**, i.e.,  $\beta$ -thalassemia and hereditary hemochromatosis
- **Iron restricted anemias**, i.e., iron refractory iron deficiency anemia (IRIDA), inflammatory diseases, some cancers, and chronic kidney disease



## Hepcidin testing challenge

A major challenge with hepcidin testing is the agreement of hepcidin concentrations between methods.

Hepcidin assay recovery ranges can span

**94% - 540%**<sup>11</sup>

# Hepcidin Kit Comparison



## Kit Summary

Validation of human hepcidin ELISA kits from five (5) manufacturers (**Table 1**) was conducted to assess:

**accuracy, precision, linearity, matrix interference, measuring range.**

Method	Manufacturer	Assay type	Range (ng/ml)	Controls Included?	Run Time 9hrs
A	Intrinsic LifeSciences (ICE-007)	Competitive	2.5 – 1000	Yes	1.45
B	BMA Biomedicals (S-1337)	Competitive	0.02 – 25	No	O/N
C	R&D Systems (DHP250)	Sandwich	0.02 - 1	No*	4.5
D	DRG International (EIA5782R)	Competitive	0.02 - 81	Yes	1.8
E	Biomatik (EKU08553)	Competitive	0.03 - 20	No	2.0

**Table 1. Human Hepcidin ELISA Kits Included in the Analysis.** Overnight, O/N. \*purchase separately



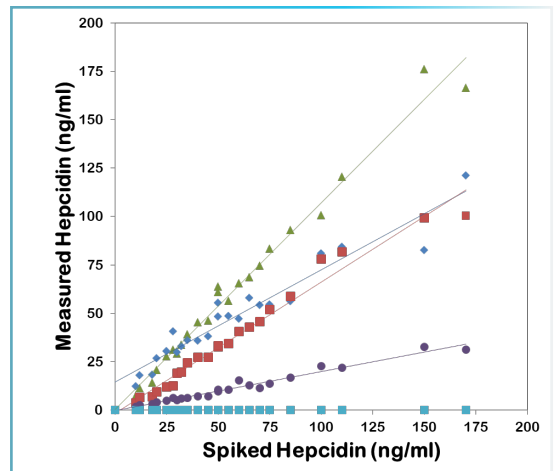
# Hepcidin Kit Comparison



## Accuracy – Spike Recovery

Methods	Slope (95%CI)	R	p
▲ A	1.07 (1.00-1.13)	0.991	< 0.001
◆ B	0.58 (0.51-0.65)	0.962	< 0.001
■ C	0.68 (0.63-0.73)	0.987	< 0.001
● D	0.20 (0.19-0.22)	0.985	<0.001
■ E	<0.001	0.021	0.923

**Table 2. Correlation statistics of serum hepcidin measured by various ELISA kits**



**Figure 1. Correlation of Serum Hepcidin by ELISA Method (Pearson correlation)**

**NOTE: A TEST WITH PERFECT ACCURACY EXHIBITS A LINEAR REGRESSION SLOPE OF 1.0**

Accuracy (**Figure 1, Table 2**) was superior using Method A, with a slope closest to 1.0 (1.07), and a 95% confidence interval (CI) that included 1.0, a trait not shared by any other method.

**Correlation coefficients (R)**, an indication of a test's ability to distinguish appropriate and proportional differences (**Table 2**), was highest for Method A but were strong for all tests, except Method E.

Method E demonstrated no ability to discriminate sample hepcidin concentrations; the test was deemed unusable and was removed from further analysis.

**Method A demonstrated greatest accuracy for detecting hepcidin across clinical ranges**

# Hepcidin Kit Comparison



## Precision

Serum samples spiked with low, medium, and high levels of hepcidin ( $n = 4$ ) were measured over two days. The average coefficient of variation (CV) within run (intra-assay) and between runs (inter-assay) was calculated.

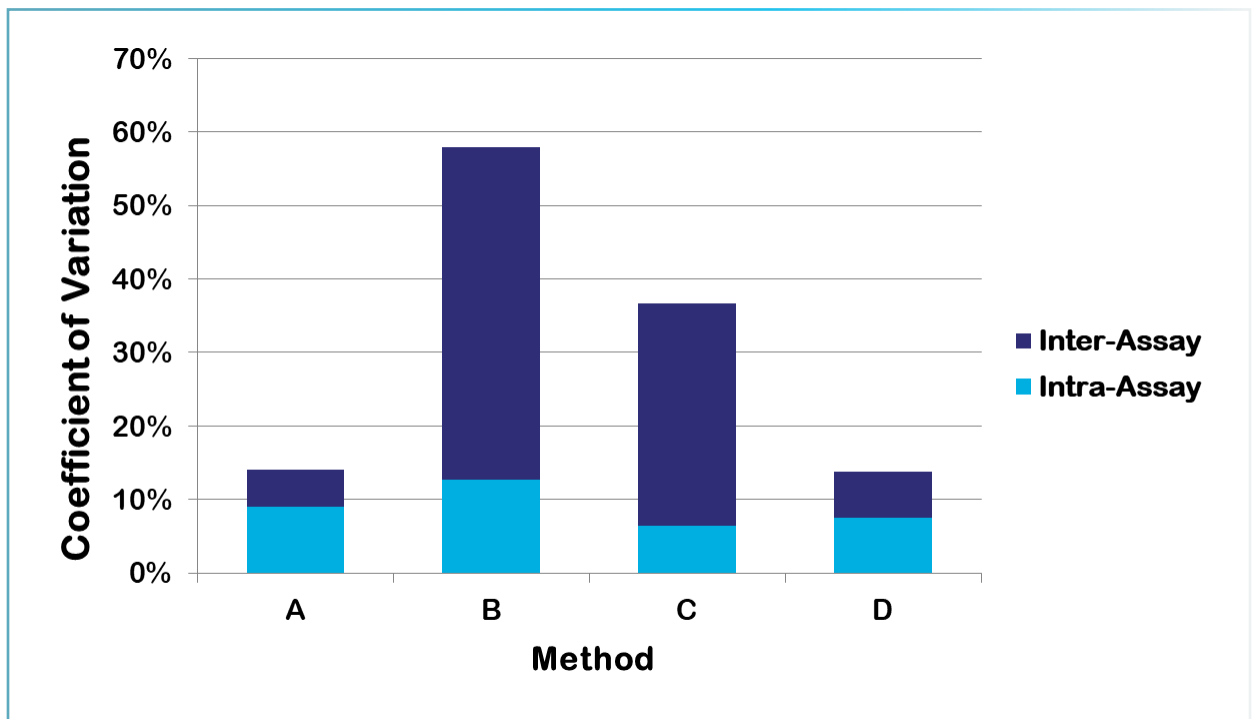


Figure 2. Intra- and Inter-assay Precision

**Methods A and D were the most precise  
with lowest total CVs of 14%**

# Hepcidin Kit Comparison



## Linearity

Method	1:2	1:4	1:8	1:16
A	3%	7%	9%	5%
B	-14%	-23%	-16%	5%
C	-3%	-9%	-8%	-11%
D	1%	3%	4%	-10%

Linearity was assessed using a high hepcidin sample diluted 1:2, 1:4, 1:8, or 1:16. The average CV across all dilutions over 2 days was calculated for each method (**Figure 3**).

Table 3. Linearity across dilutions, relative error.

Method C demonstrated the lowest CVs,  
closely followed by D and A.

Cumulative CVs of < 10% achieved by all methods, except Method B.

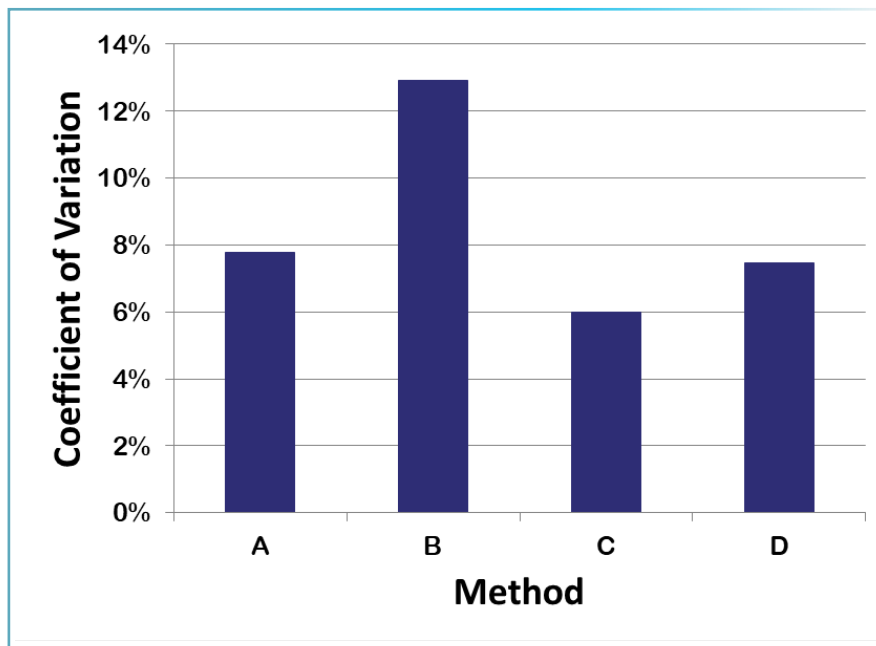


Figure 3. Average coefficient of variation for linearity across all dilutions

# Hepcidin Kit Comparison



## Matrix Effect

The average relative error of sample type for hepcidin was calculated from Li Heparin and EDTA plasma samples compared to serum (n = 5).

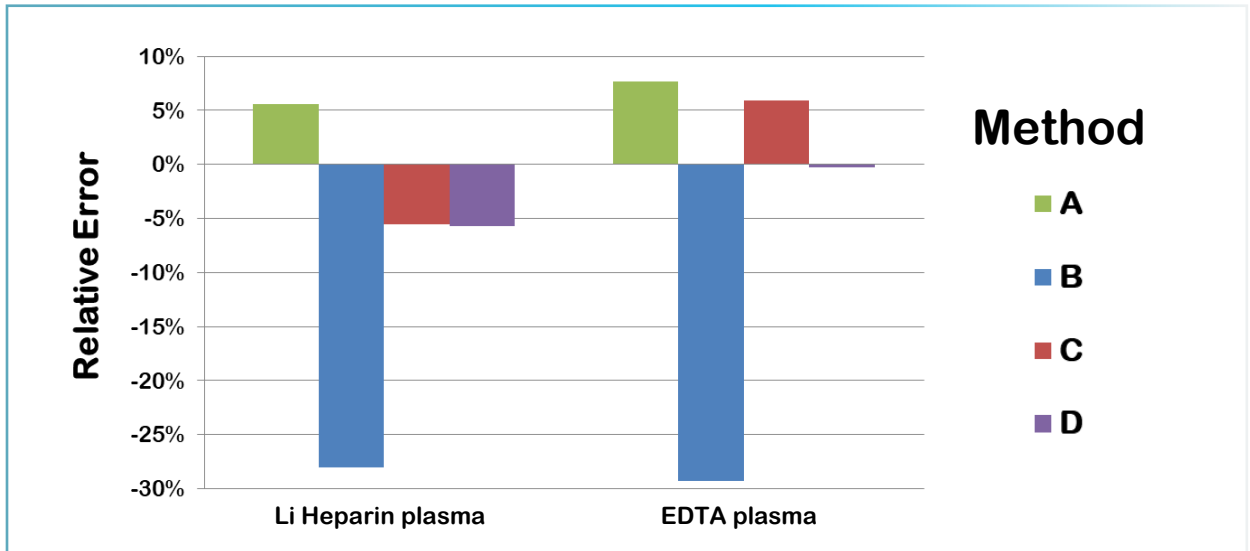


Figure 4. Plasma vs Serum, Relative Error

Methods A, C, and D showed no matrix effect across sample types: serum, EDTA plasma, and Li heparin plasma

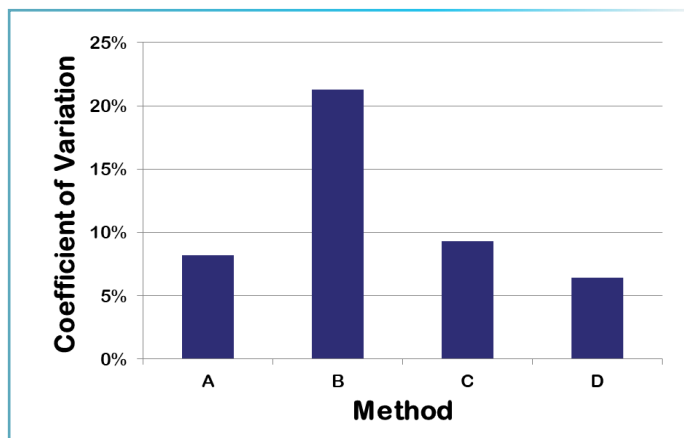


Figure 5. Serum and Plasma Matrix Precision, CV.

The average coefficient of variation (CV) across all three matrices (n = 5) was <10% in 3 of 4 methods. Method B was most affected by use of plasma samples.



# Conclusion



## At-a-glance

Method	Accuracy	Precision Intra- And Inter- assay	Linearity	Sample Matrix	Measuring Range	Limit Of Quantitation
A	✓✓	✓✓	✓	✓	✓✓	
B						
C			✓✓	✓		✓*
D		✓✓	✓	✓✓		
E		n/a	n/a	n/a	n/a	n/a

✓✓ optimal

✓ suitable (depending on intended research)

\*While assay sensitivity can be useful in research, relevance of a low hepcidin limit of quantitation should be evaluated based on physiological and clinical properties of the individuals being evaluated in the study.

A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R)

# Conclusion



## Assay Range

The best assay will span the normal range encompassing the diagnostic limit for iron deficiency on the low end with room to spare on the high end to accommodate cases of inflammation.

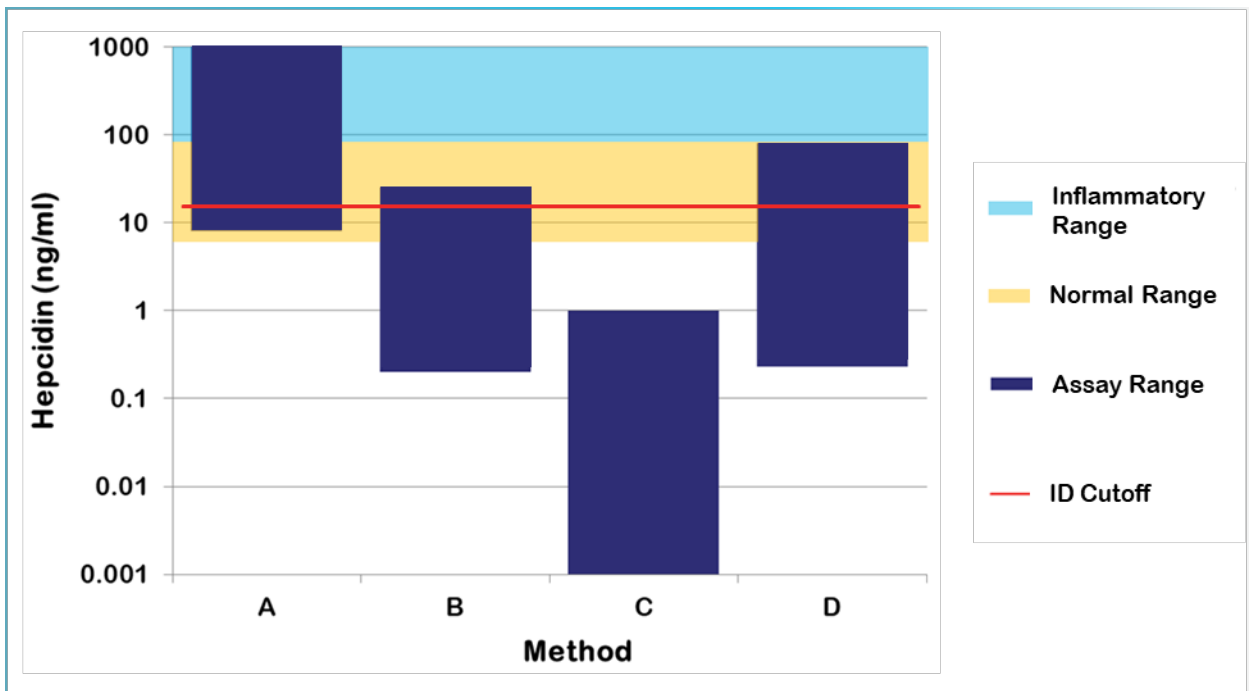


Figure 6. Assay Range. ID, iron deficiency.

The hepcidin normal range is 6.2 – 82.6 ng/ml (5-95%). Hepcidin predicts iron deficiency below 15 ng/ml<sup>1</sup>.

**Method A covers the entire clinical range without the need to dilute samples saving time and money.**

# Conclusion



## Summary Table

	Accuracy	Precision intra- and inter-assay	Linearity
Optimal Method By Category	<b>A</b>	<b>A, D</b>	<b>C</b>
Ideal hepcidin kit properties	Test slope of 1.0 indicates results of two test methods produce the same result.	Precision is an indicator of the assay reproducibility. CV's < 10 are considered acceptable in a clinical setting; < 5 would be considered highly reproducible.	Linearity is the accuracy of a measurement from a diluted sample. Typically accuracy declines as sample dilution increases.
Why important? What is impact on research/data?	Adjusting to accommodate a slope that deviates from 1.0. requires time, reagent and samples.	Low within-run (intra- assay) AND between run (inter-assay) CVs help minimize variability of results.  An assay with a poor precision can increase variability and widen confidence intervals, weakening the strength of the data and lowering scientific merit.  Low intra-assay CVs can be diminished by high inter-assay CVs.	If a sample needs to be diluted, the accuracy of the measurement is dependent on the linearity of the assay.  An assay with a narrow range AND poor linearity will hinder accurate measurement and likely affect the relationships between study groups. It is best to minimize large sample dilutions to reduce sources of analytical error.

A, Intrinsic LifeSciences (ICE-007); B, BMA Biomedicals (S-1337); C, R&D Systems (DHP250); D, DRG International (EIA5782R)



The leading developer of  
immunoassays for human hepcidin

Since 2006, **Intrinsic LifeSciences (ILS) assays** have been the method of choice for hepcidin testing in more than 130 research projects and clinical trials.

ILS operates **IntrinsicDx**, the only CLIA certified, CAP accredited U.S. laboratory performing clinical hepcidin immunoassay testing.



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