

Worldwide harmonization of serum hepcidin assays

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Background

Absolute serum hepcidin concentrations measured by various methods differ considerably, complicating interpretation of results and rendering reference intervals method-dependent. A previous hepcidin harmonization study identified a commutable secondary reference material (sRM).

Aim

We aim for a **higher level of equivalence of worldwide hepcidin measurement procedures** by i) validating the commutability of the sRM and its functionality to increase the degree of equivalence between measurement procedures (i.e. harmonize methods) and ii) producing a large batch of 2 levels of this sRM for international use, making worldwide harmonization possible.

Design

A large batch of two levels reference material, consisting of lyophilized serum with cryoprotectant, was produced and validated in terms of commutability. A value to the sRM was assigned. We applied technical procedures developed by the International Consortium for Harmonization of Clinical Laboratory Results to ensure harmonization potential.

Samples

- Native individual serum samples (n=16)
- Serum pools (n=8)
- Candidate reference material (cRMs, 2 levels)

Assays

Samples were shipped to 9 laboratories worldwide, representing 4 mass spectrometry- and 5 immunochemical assays (MS or IC).

Measurements and analyses

- Laboratories measured triplicates of all samples within one run.
- Analytical performance, current- and achievable equivalence were assessed with results of 16 individual serum samples and serum pools.

Commutability and value assignment

We previously validated the commutability of the sRM according to the Clinical and Laboratory Standards Institute (CLSI) C53-A protocol. For the current study commutability was confirmed using regression analysis, which showed that the mean of triplicate measurements of calibrator samples fell inside the 95% confidence interval of the regression line based on the mean of individual native samples for each method (Y-axis) and the mean of all methods (X-axis).

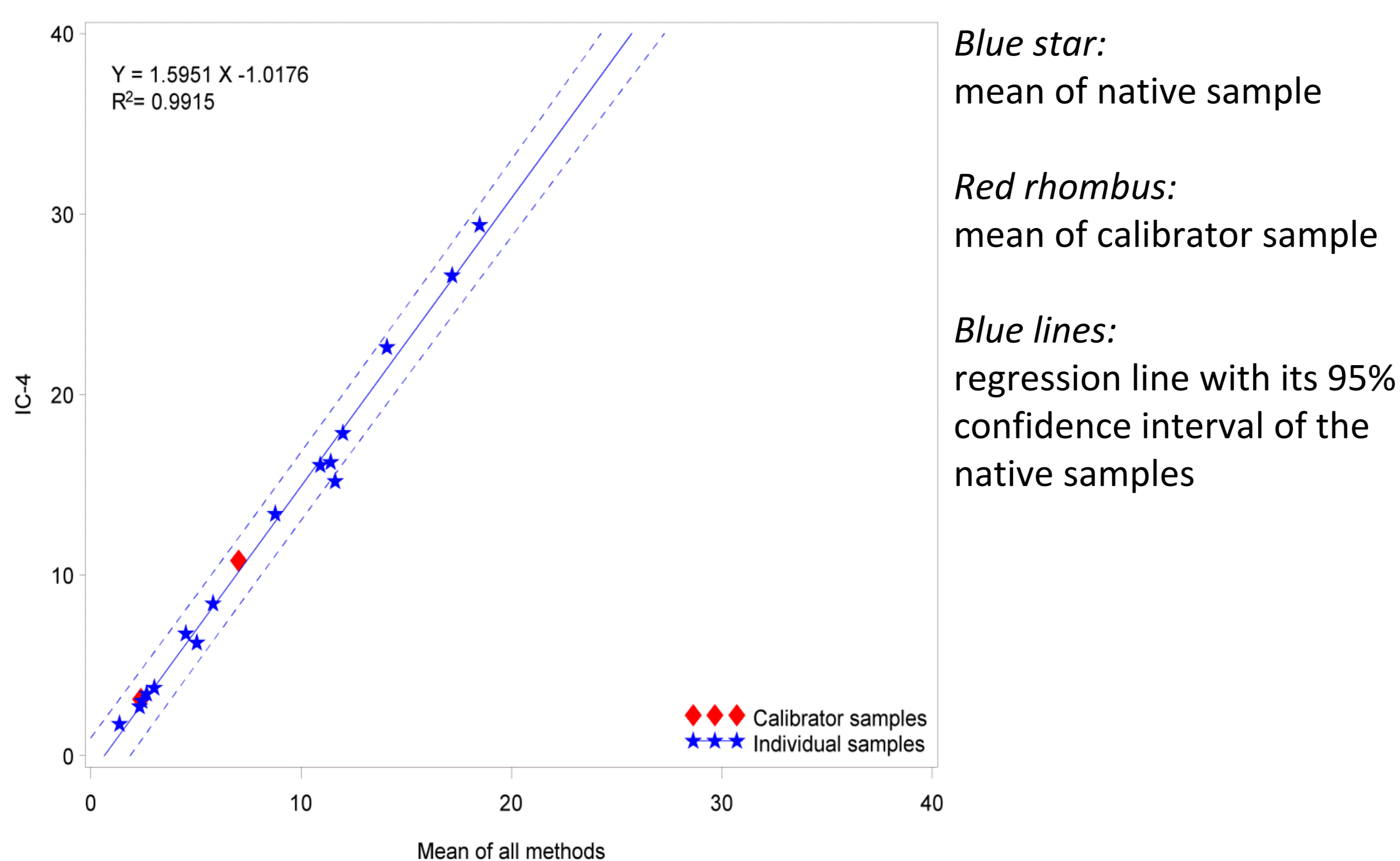


Figure 1. Representative commutability plot of one method (IC-4) against the mean of all methods.

We assigned a value to the sRM using our own candidate reference method and a consensus approach including 9 worldwide validated methods.

| | Assigned value (SD) by hepcidinanalysis.com [nmol/L] | Mean value (SD) of 9 validated methods worldwide [nmol/L] |
|--------------|--|---|
| Low level | 0.851 (0.049) | 2.38 (1.22) |
| Middle level | 3.758 (0.090) | 7.03 (3.15) |

Degree of equivalence

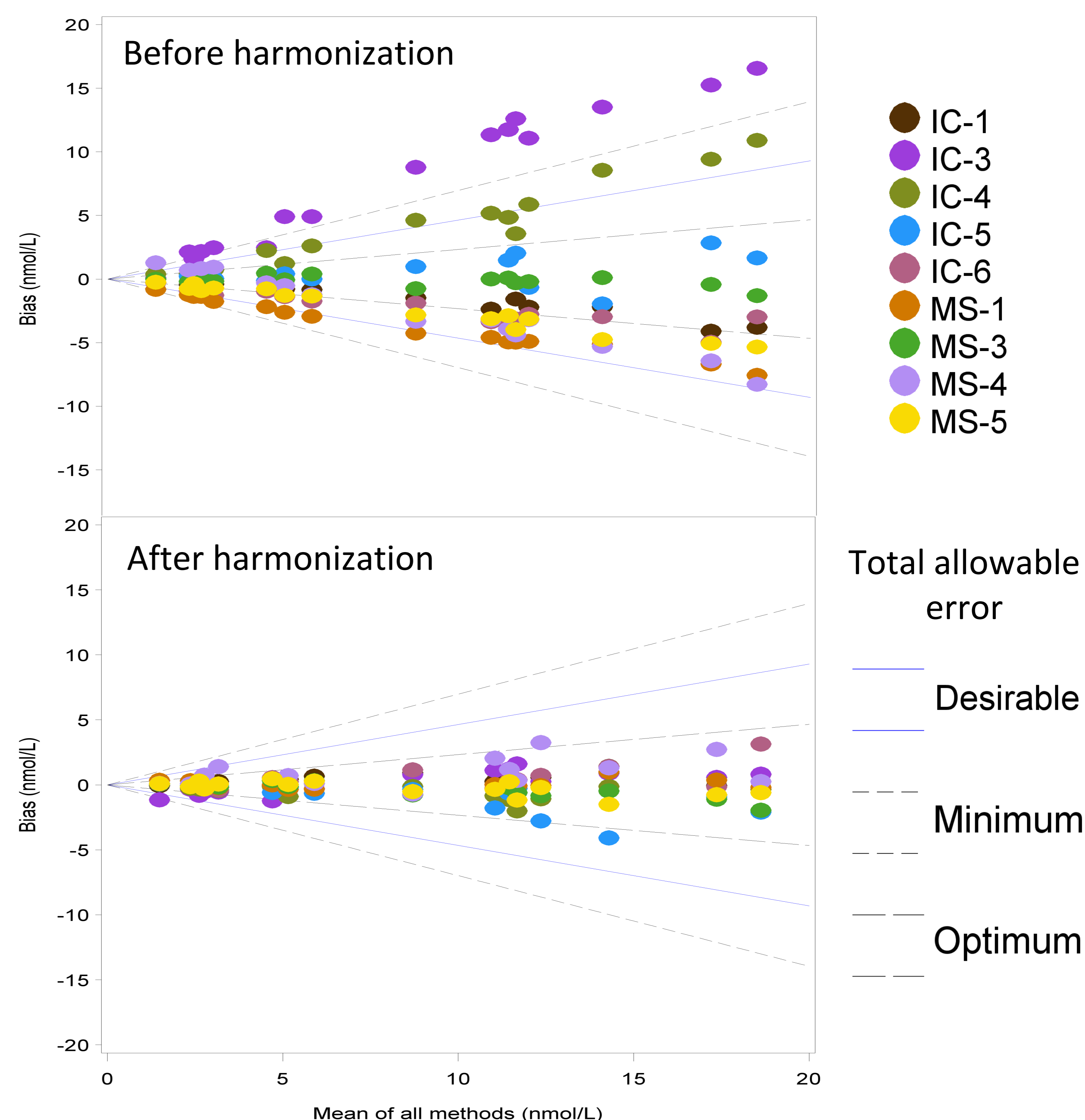


Figure 2 . Degree of equivalence expressed as total allowable error (TEa) before and after calibration with native lyophilized serum with CLP. Data points above each other represent measurements of the 16 samples by all different assays. Because of the absence of a true value, the x-axis represents the mean results of the samples for all methods. The y-axis shows the bias, i.e. the difference between results of individual samples of each method and the mean of all methods. The lines represent limits for optimum, desirable and minimum TEa.

Summary and conclusions

- A functional two-level secondary reference material has been produced**, with its value assigned through inter-laboratory consensus.
- We validated commutability of this secondary reference material.
- By mathematical simulation we showed **harmonization of hepcidin assays is a possibility in the future.**
- We created a large batch of sRM that allows worldwide harmonisation of hepcidin assays.
- Studies are ongoing to:
 - validate a primary reference material to allow future full standardization of hepcidin assays;
 - determine whether worldwide laboratory harmonization has been achieved.

Reference

van der Vorm L, Hendriks J, Laarakkers C, Klaver S, et al Toward Worldwide Hepcidin Assay Harmonization: Identification of a Commutable Secondary Reference Material. *Clinical Chemistry*. 2016 Jul;62(7):993-1001.