Standardization of insulin: challenges and solutions

Standardization of protein biomarker measurements: New initiatives for reference measurement systems

Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)
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Overview

Challenges & solutions

- **Certified Reference Material**
- **Reference method & Reference laboratories**
- **Standardization model**
- **Organizational structure**
- **Implementation**

American Diabetes Association (ADA) Insulin Standardization Workgroup
European Association for the Study of Diabetes (EASD)
International Federation for Clinical Chemistry and Laboratory Medicine (IFCC)

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Human insulin reference material

Current status
WHO distributes
– **Insulin IS 83/500 & IRR 66/304**
– **C-peptide IRR 84/510**
– **Proinsulin IRR 84/611**
Content assignment based on biological assays (IU's)

Compendial Agencies (USP, PhEur, JP) moved away from having 1 'global' reference standard and started to officially distribute independently produced & characterized *biosynthetic* reference standards
Content assignment: see monographs in Pharmacopeia

Manufacturers have their in-house reference standard that is shown equivalent to the compendial standards

Human insulin reference material

ADA Workgroup – Phase III study
Pure human insulin (biosynthetic) was donated by Novo Nordisk. The purity was 92.8% (mass fraction); this value was derived from estimating the mass fractions of inorganic impurities (residual sulphated ash), water and specific insulin variants detectable by HPLC (internal certificate from Novo Nordisk).

Material of excellent quality!

But, the Novo Nordisk certificate was not official!

Miller WG, Thienpont LM, Van Uytfanghe K, Clark PM, Lindstedt P, Nilsson G, Steffes MW; Insulin Standardization Work Group. Toward standardization of insulin immunoassays. Clin Chem 2009;55:1011-8.

Human insulin reference material

Future directions

Request to the Expert Committee on Biological Standardization (ECBS) of the WHO to implement

– New International Reference Standards for biosynthetic human insulin/C-peptide

– Certified property value using the SI-system (mass units of mgs)

Note: conversion factor between activity units and mgs is well agreed (1 IU = 0.0347 mg)

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Reference method

Isotope dilution

–Liquid chromatography

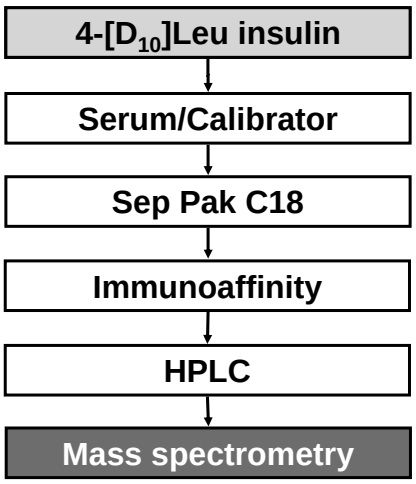
/Tandem mass spectrometry

(ID–LC/Tandem MS)

Calibrated with NOVO insulin standard

Van Uytfanghe K, Rodriguez-Cabaleiro D, Stöckl D, Thienpont LM. New liquid chromatography/electrospray ionisation tandem mass spectrometry measurement procedure for quantitative analysis of human insulin in serum. Rapid Commun Mass Spectrom 2007;21:819-21.

UGent procedure



Performance specifications

ADA Workgroup

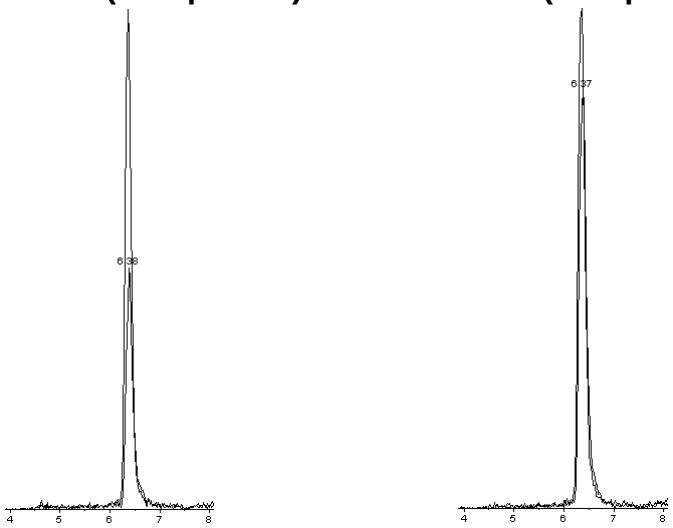
- **Limit of quantitation (LoQ): 12 pmol/L**
- **Total CV limit: 3% (6 – 7% at the LoQ)**
- **Bias limit: ±5%**

We succesfully validated our ID-LC/tandem MS procedure according to those specifications and applied it in a pilot study

Rodriguez-Cabaleiro D, Van Uytfanghe K, Stove V, Fiers T, Thienpont LM. Pilot study for the Standardization of Insulin Immunoassays with Isotope Dilution-Liquid Chromatography/Tandem Mass Spectrometry. Clin Chem 2007;53:1462-9.

Chromatograms

Selected chromatograms
Donor #25 (12.1 pmol/L) and Donor #02 (23.5 pmol/L)



Reference laboratories

Ghent University (Belgium)
Faculty of Pharmaceutical Sciences
Laboratory for Analytical Chemistry
Director: L. Thienpont

Albert Einstein College of Medicine, New York (USA)
Department of Internal Medicine
Division of Endocrinology & Metabolism
Director: D. Stein
(candidate reference measurement procedure under development)

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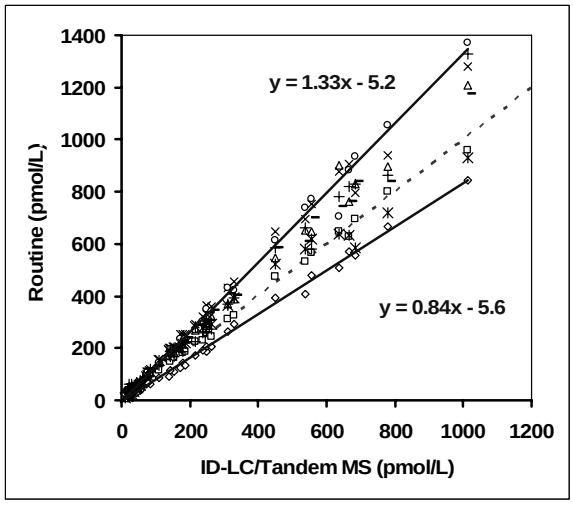
Status at start: trueness & comparability



- **Mean of all assays close to ID–LC/tandem MS**
- **Factor between assays: 2.6 (<50 pmol/L); 1.6 (>50 pmol/L)**

Materials qualifying for standardization

ADA Workgroup – Phase III study

- **Insulin standard: PRMI-2***
- **Serum pools**
- **Native sera from single donations**

***Phase I**

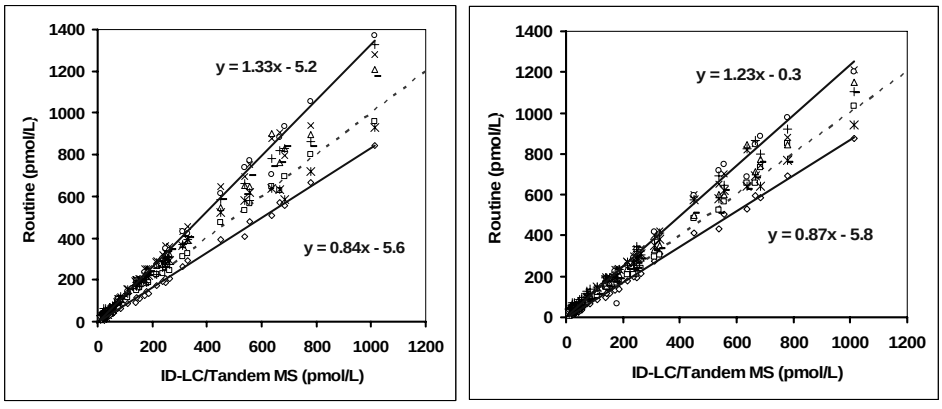
Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, et al. Report of the American Diabetes Association's task force on standardization of the insulin assay. Diabetes 1996;45:242–5.

Phase II

Marcovina S, Bowsher RR, Miller WG, Staten M, Myers G, Caudill SP et al.; Insulin Standardization Workgroup. Standardization of insulin immunoassays: report of the American Diabetes Association Workgroup. Clin Chem 2007;53:711-6.

Recalibration with pure insulin (PRMI-2)

Manufacturer **PRMI-2**



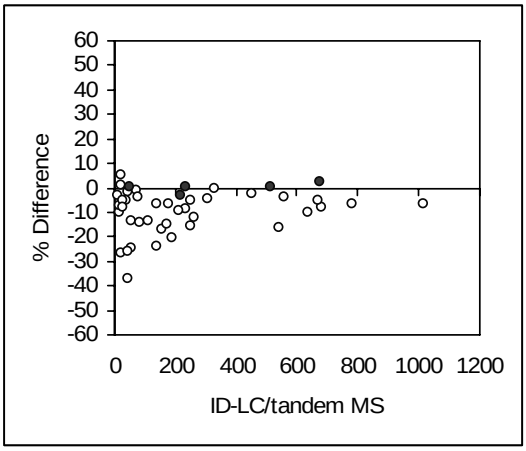
Minor improvement

>PRMI-2 not commutable

Recalibration with pools

Assay A weighted Deming (mean results)

	Slope	Intercept (pmol/L)
Pools	**0.892**	**-1.3139**
Samples	**0.798**	**-1.1994**

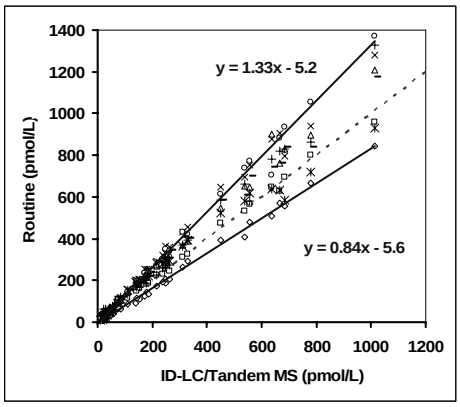


% Difference after recalibration (pools in red)

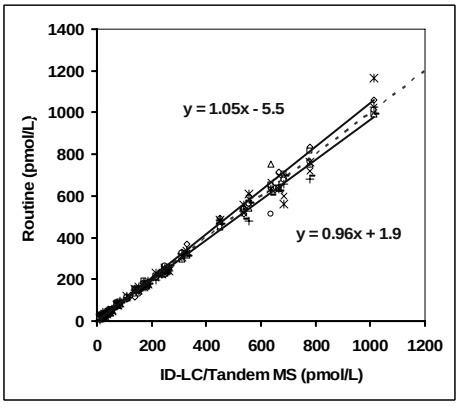
Pools not commutable for certain assays

Recalibration with single donations

Manufacturer



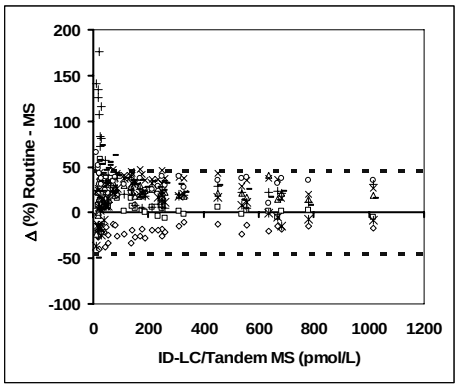
Single Donation

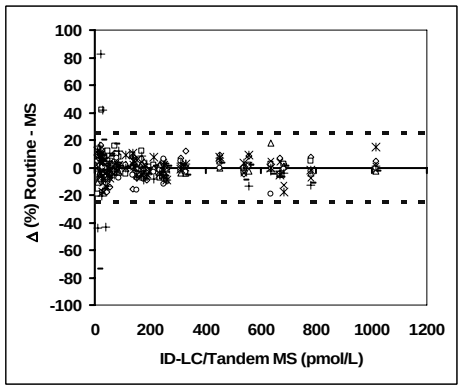


Major improvement

Recalibration with single donations

Manufacturer **Single Donation**



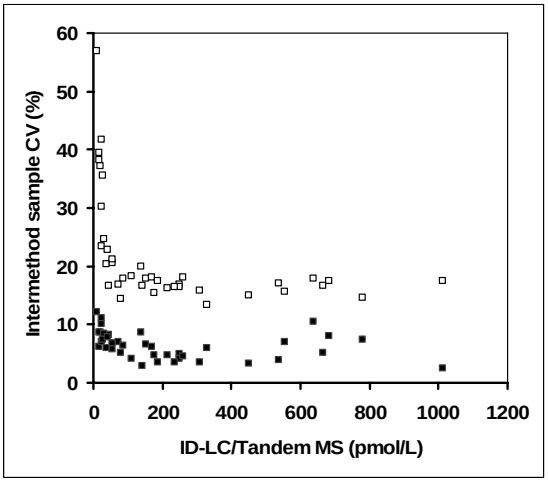


Major improvement

>Spread entirely within-assay

Recalibration with single donations

Intermethod sample CV
Before recalibration: □
After recalibration: ■ (outliers removed: 1B, 4F, 1G, 1H)



Chosen standardization model

Method comparison with native sera

Standardization

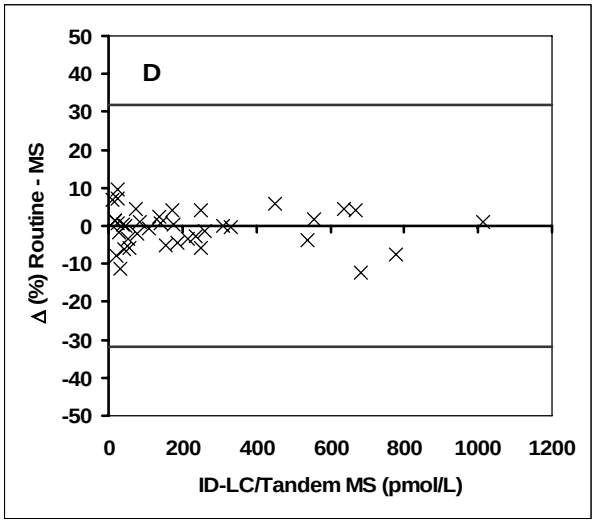
- **Iterative model**
- **Basic investigations with single donation normal sera**
- **Final recalibration with single donation sera covering the complete measurement range**
- **Role of pools is still under discussion**

Quality assessment

Single donation sera

“No standardization without assessment of quality”

Quality assessment of immunoassays



Generally good for normal sera: fulfil the TE limit of 32%

Practical aspects

Iterative process

1. **High-volume panel (~180-200 mL/serum) from healthy donors (<CLSI C37-A); assigned by RMP**
   - **3 mL/sample/assay**
   - **3 different reagent/calibrator lots; IQC**
   - **include master calibrators for recalibration**

2. **Low-volume panel of clinical samples (~10 mL/sample) (30 L, 30 N, 30 H) (<usual blood collection tubes); assigned by RMP**
   - **0.5 mL/sample/assay**
   - **include samples from panel 1**
   - **1 batch, 2 replicates**
   - **include master calibrators for recalibration, own pools for 'long-term stability'**

3. **Repetition of step 2 (refinement; select 3 'best' assays)**

Personal view!

Practical aspects

'Continuity' panel (low-volume panel clinical samples, ~10 mL/sample, 30 L, 30 N, 30 H)
- **assigned by 3 'best' assays**
- **0.5 mL/sample**
- **1 batch, 2 replicates**
- **include master calibrators**

Caveat!
'New' panels should always be developed in overlap with 'previous' one

Personal view!

Practical aspects

IFCC

- **Infrastructure for all projects**
- **Clinical samples**
- **Bio-banking**

Personal opinion!

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Organization

American Diabetes Association Insulin Standardization Workgroup

in cooperation with

European Association for the Study of Diabetes (EASD)

International Federation for Clinical Chemistry and Laboratory Medicine (IFCC)

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

International Diabetes Federation (IDF)

Organization

Global Standardization Workgroup

- **Insulin**
- **C-peptide**
- **Proinsulin**

Personal opinion!

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Implementation

Requirements

- **Stable infrastructure**
- **Internationally coordinated**
- **Professionally managed**
- **Clinically supported (clinical societies)**
- **Standardization in a concerted way (cf. creatinine)**