Commutability studies undertaken by the LNE : the case of lipid and lipoprotein testing

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MEASUREMENT & STANDARDS

Keys to COMPETITIVENESS and A SAFER WORLD

Traceability in laboratory medicine : regulatory drivers

Reform of medical biology in France

By 2016-2020, accreditation according to ISO 15189 will become mandatory for ALL clinical laboratories (both public and private)

In vitro diagnostic Directive on medical devices 98/79/EC

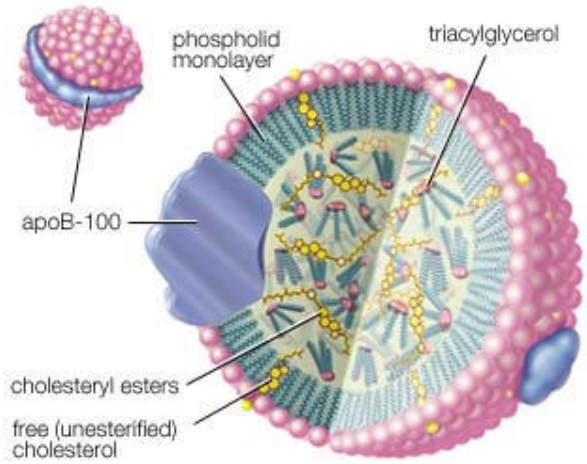
« The traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order »

- **Development of reference methods for the main biomarkers used in clinical biology : creatinine, glucose, HbA1c, TCh, LDL-C, HDL-C, TG, …**
- **Production of Certified Reference Materials**
- **Assignment of reference values to calibration & quality control materials**

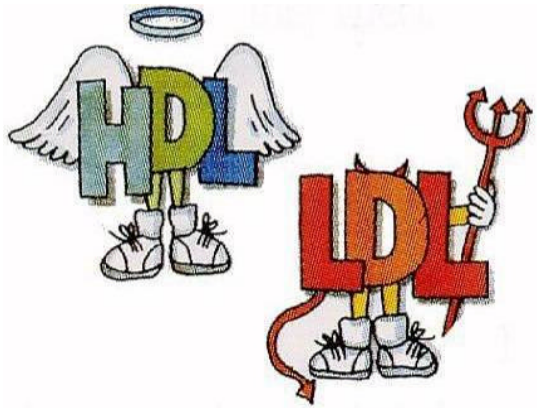
Why reliable lipid / lipoprotein testing is important

Lipid profile : Total Cholesterol + LDL-C + HDL-C + Triglycerides

- ü **Assessment of CVD risk**
- ü **7th most common analysis performed in French clinical labs (16 million tests / year)**
- ü **2nd most expensive analysis for the French health insurance (> 150M€ / year)**



- ü **Costs related to reimbursement of statins > I B€ / year**
- ü **French court of auditors shown that 500M€ could be saved with a better therapeutic management**



à **Need for reliable diagnostic tests**
… and efficient quality assessment surveys to ensure post-market Vigilance

LNE's activities in lipid/lipoprotein testing

ü Validation of higher order reference methods

1/ Publication of method(s) validation in peer review journals

Clinical Biochemistry 46 (2013) 359–364

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Validation of a reference method for total cholesterol measurement in human serum and assignation of reference values to proficiency testing samples

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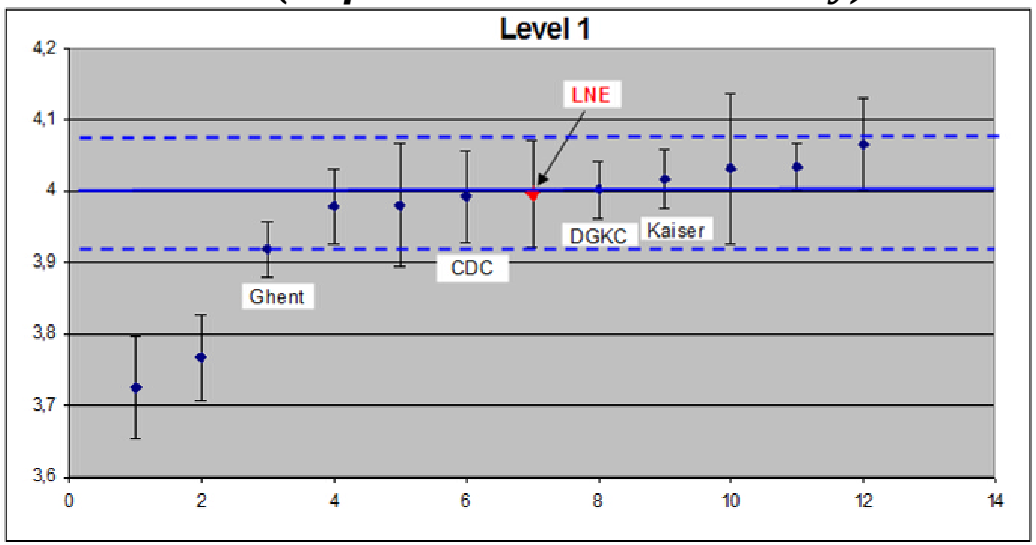
LNE's activities in lipid/lipoprotein testing

ü **Validation of higher order reference methods**

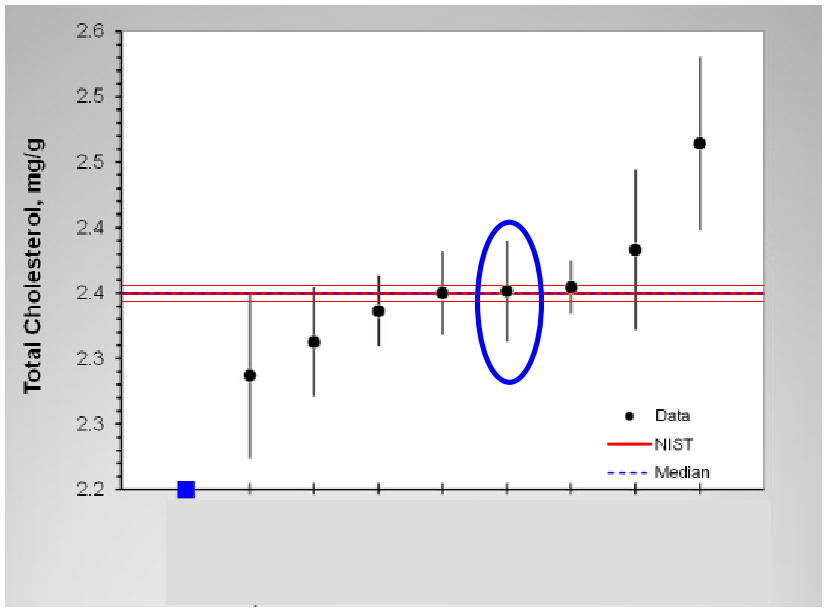
1/ Publication of method(s) validation in peer review journals

2/ Participation to international comparisons (CCQM, IFCC RELA)

2011 IFCC RELA comparison on TCh (Reference Laboratories only)



2013 CCQM comparison on TCh (National Metrology Institutes)



LNE's activities in lipid/lipoprotein testing

ü Validation of higher order reference methods

1/ Publication of method(s) validation in peer review journals

2/ Participation to international comparisons (CCQM, IFCC RELA)

3/ Accreditation according to ISO 17025 and ISO 15195

4/ LNE recognized as Reference measurement service by the JCTLM

BIPM
Bureau International des Poids et Mesures

Database of higher-order reference materials, measurement methods/procedures and services

JCTLM Database
Laboratory medicine and *in vitro* diagnostics

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Analyte	total cholesterol
Material or matrix	blood serum, calibration solution
Applicable material or matrix	lyophilized, fresh, or frozen human serum, calibration solution
Quantity	Amount-of-substance concentration
Service measurement range	1 mmol/L to 10 mmol/L
Expanded uncertainty (level of confidence 95%)	3 % to 1 % The expanded uncertainty is relative.
Interlaboratory comparison results	RELA - IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine at http://www.dgkl-rfb.de:81/index.shtml
Measurement principle	ID-GC/MS

Assignment of reference values to PT materials

European standard

French standard

NF EN ISO 15189
August 2007

Classification index: **S 92-060**

Medical laboratories

Particular requirements for quality and competence

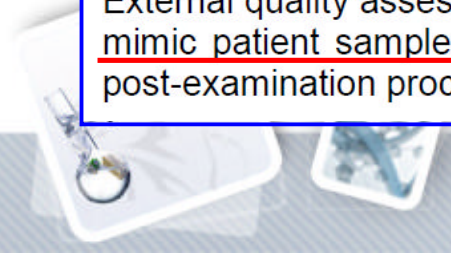
5 Technical requirements

5.6 Assuring quality of examination procedures

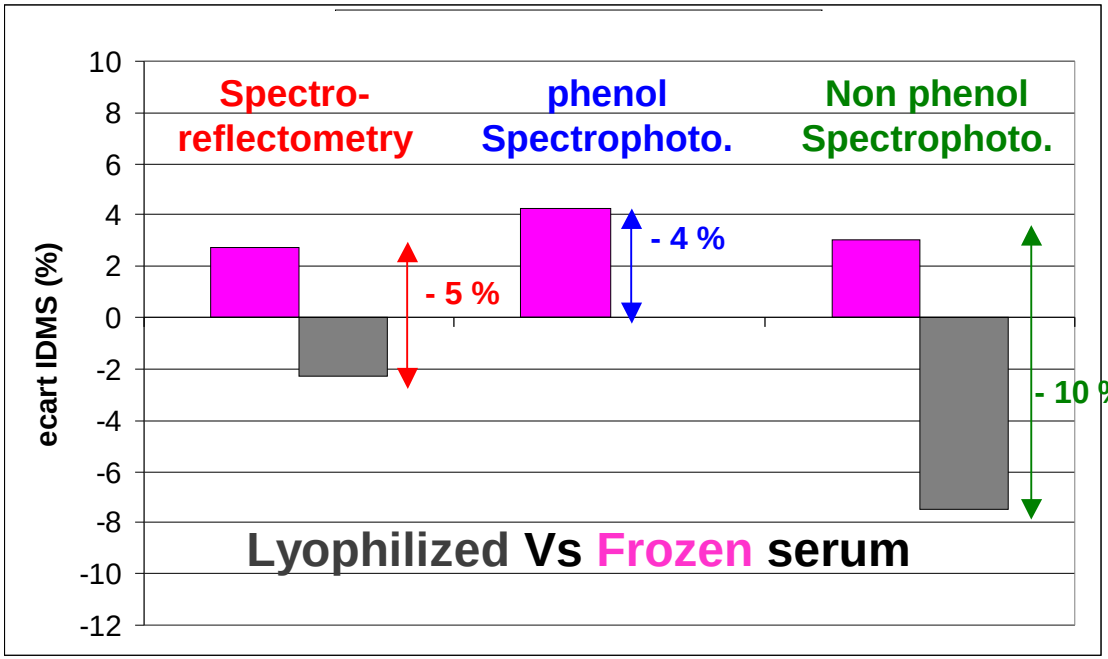
5.6.3 A programme for calibration of measuring systems and verification of trueness shall be designed and performed so as to ensure that results are traceable to SI units or by reference to a natural constant or other stated reference. Where none of these is possible or relevant, other means for providing confidence in the results shall be applied, including but not limited to the following:

5.6.4 The laboratory shall participate in interlaboratory comparisons such as those organized by external quality assessment schemes. Laboratory management shall monitor the results of external quality assessment and participate in the implementation of corrective actions when control criteria are not fulfilled. Interlaboratory comparison programmes shall be in substantial agreement with ISO/IEC Guide 43-1.

External quality assessment programmes should, as far as possible, provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre- and post-examination procedures.



Why commutability matters



Measurement error sources

- Random analytical variation within runs
- Random variation between runs
- Bias of the method
- Sample-specific error

METHOD

SAMPLE

- To rigorously assess field methods trueness, PT samples should be commutable!
- Calibrators should also be commutable, otherwise, the traceability chain is broken!
- As a material can be commutable for a given method but not for another one, commutability should be evaluated for ALL field methods !

LNE's commutability study

<u>Objective</u> : Qualify 2 candidate CRMs and 9 PT samples intended to be used as trueness controls

ü LNE's candidate CRMs : LNE CRM BIO 101a

- à **The 1st French CRM for clinical biochemistry markers**
- à **2 pools of Human Frozen serum (1000 x 1mL each)**
- à **Prepared according to NCCLS-C37A @ Solomon Park**
- à **2 levels of concentration (one low, one high)**
- à **Glucose, creatinine, TCh, LDL-C, HDL-C & TG**

ü 9 PT samples from various EQAS

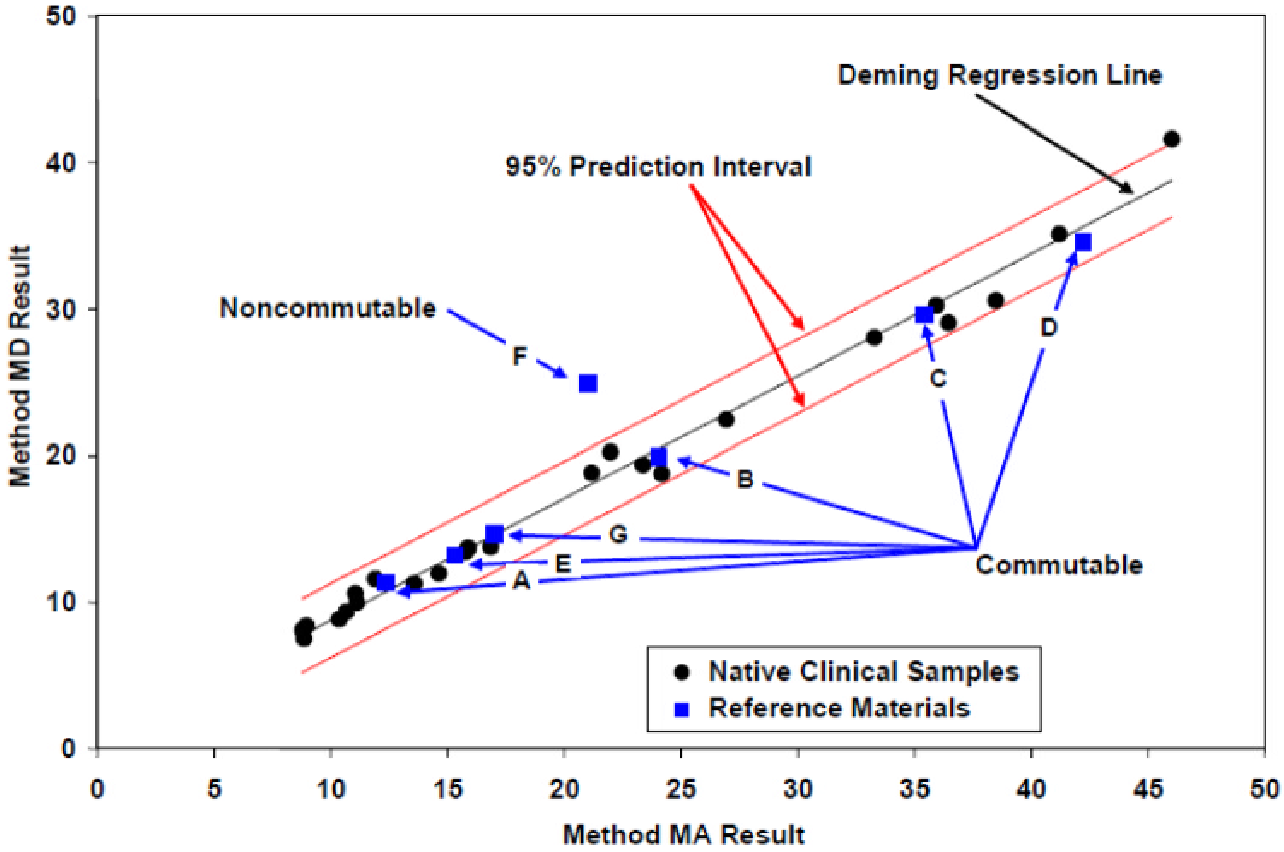
- **5 PT samples from the French mandatory EQAS (Lyophilized serum)**
- **3 PT samples from an EQAS in Singapour (Frozen serum - NCCLS C37-A)**
- **1 PT sample from a French voluntary EQAS (Frozen serum)**

Commutability assessed for the most popular methods à 37 clinical labs

7 Roche Cobas, 6 Siemens Vista, 6 Abbott Architect, 5 Beckman DxC, 3 Ortho-CD Vitros, 3 Beckman AU, 2 Siemens Advia, 2 Roche Modular, 2 Thermo KoneLab

Commutability assessment according to CLSI C-53A

CLSI C53A Guidelines : analyze with 2 different methods the samples whose commutability should be assessed along with at least 20 native samples

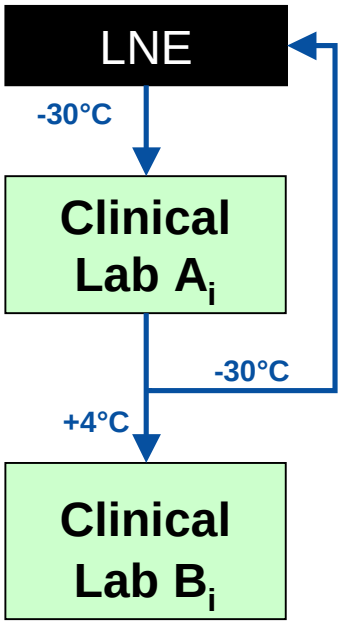


1) Native samples à Linear regression à 95% prediction interval

2) Sample is commutable if it falls within the prediction interval

Study design : participants, samples and logistics

Design 1 : 15 Pairs of laboratories (15 group A + 15 group B)



X15

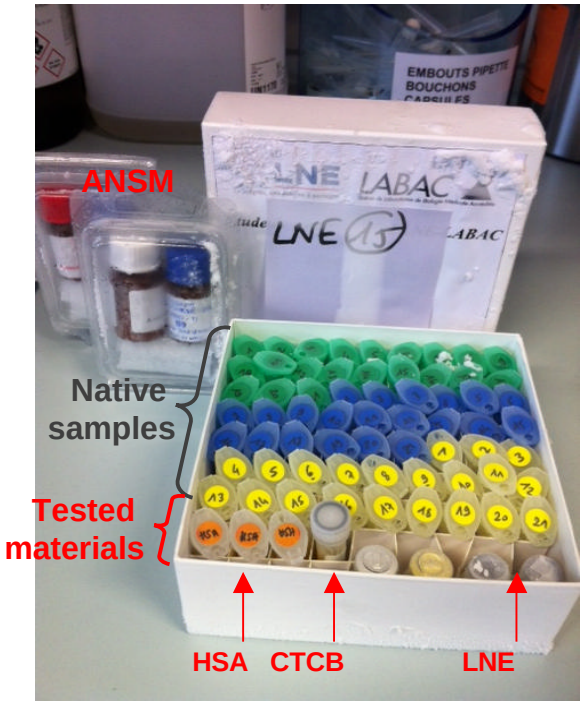
1/ CRMs & PT samples shipped to Group A labs that had to :

2/ Select 21-25 fresh clinical samples that were collected the same day (as function of their concentration),

3/ Aliquote serum into 3 fractions,

4/ Analyze all samples in triplicate in the same analytical run,

5/ Ship back all materials to LNE and to Lab B for analysis



Ø Commutability assessed for only 2 methods at the same time

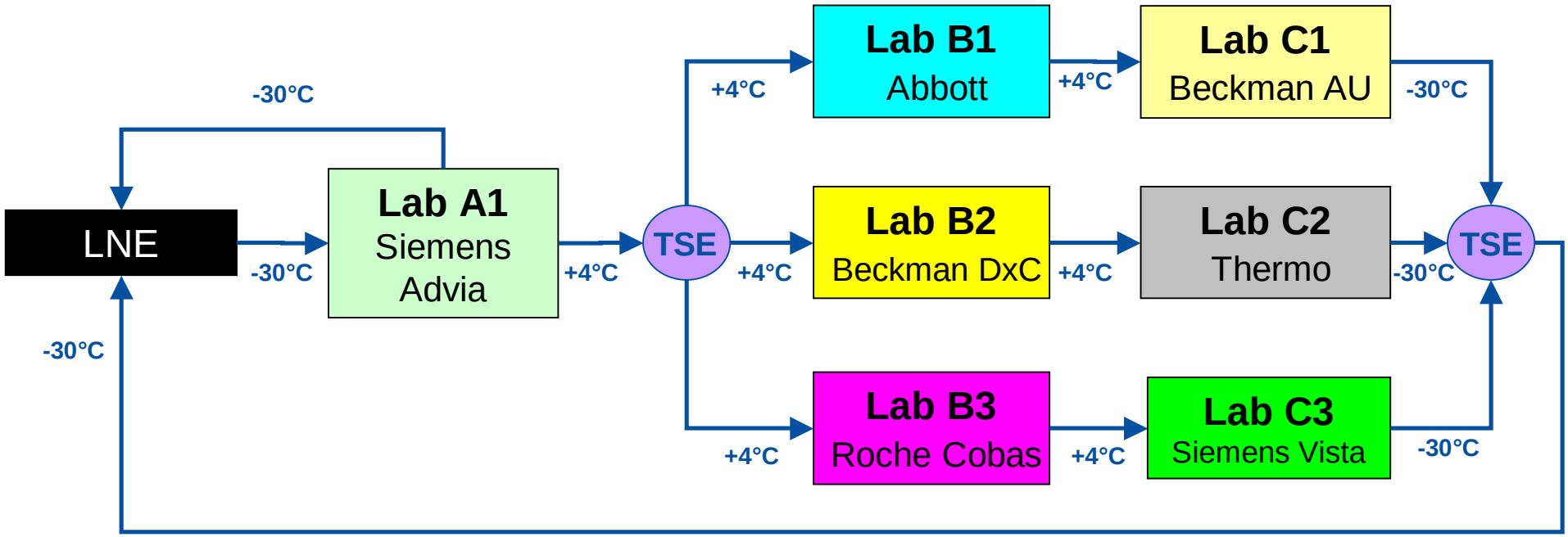
à Need to involve a high number of laboratory pairs : labor intensive

Ø Participants didn't analyze the same set of clinical samples

à Potential troubles when it comes to compare results together

Study design : participants, samples and logistics

Design 2 : 1 Group A lab + 3 Group B labs + 3 Group C labs

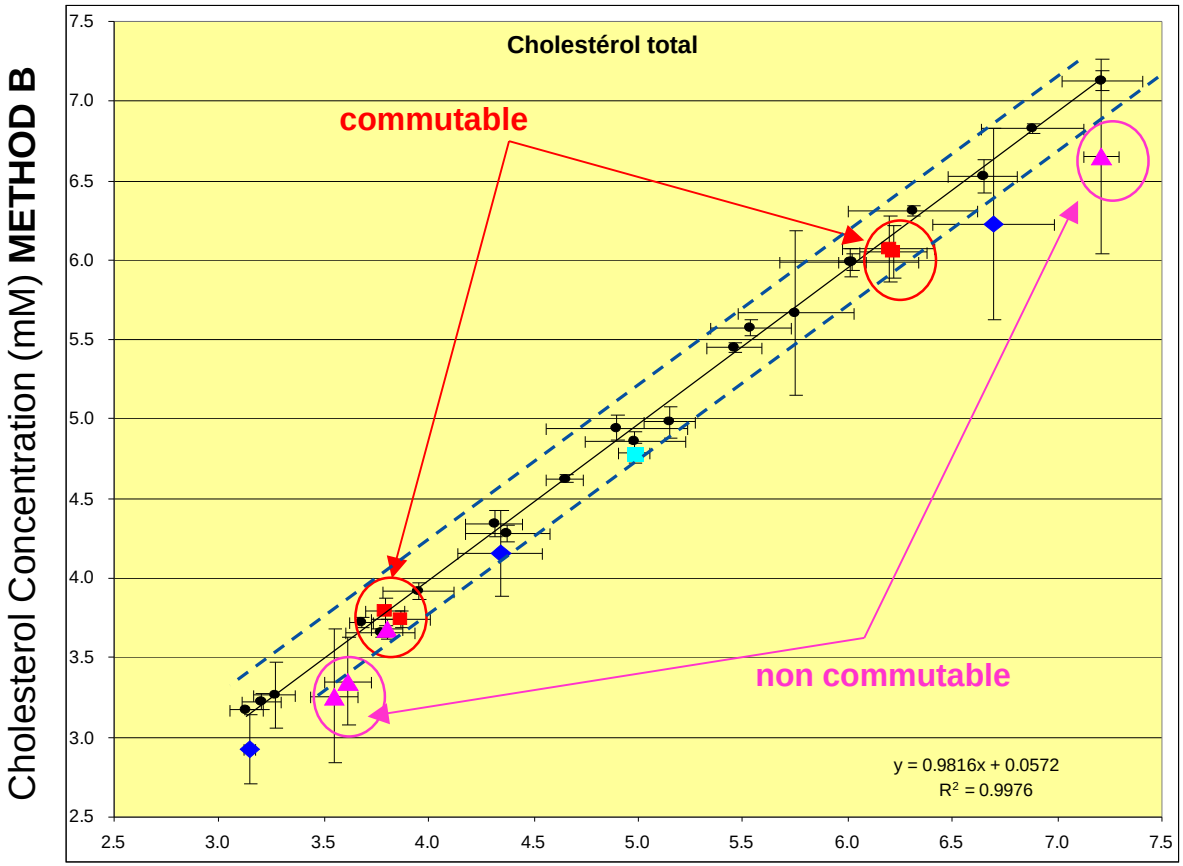


Multiplexing commutability : simultaneous assessment of

11 materials, 5 parameters, 7 manufacturers

Limitations : sample volume available, tricky logistics

LNE's commutability study : the example of TCh



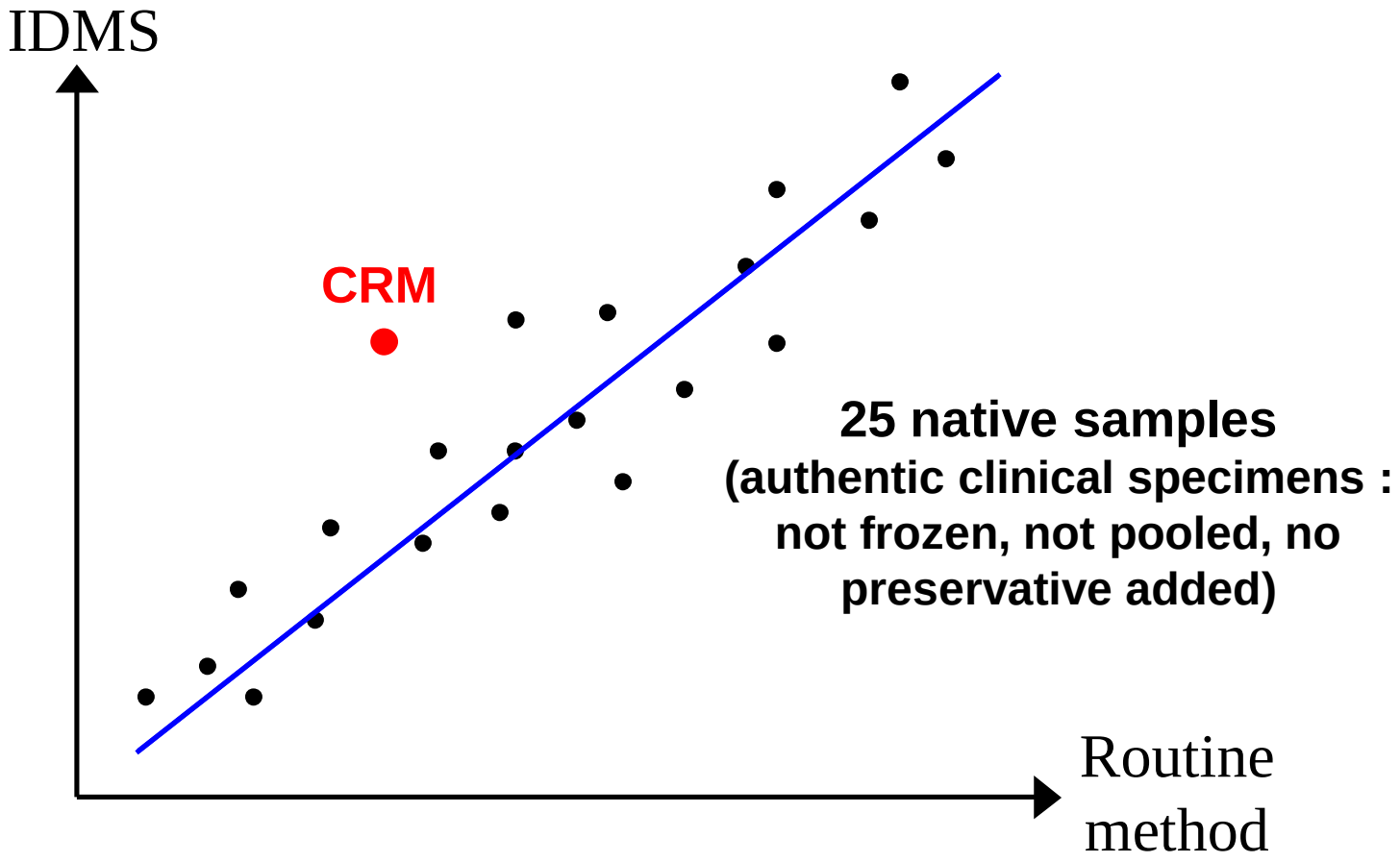
Labo B	Cholestérol total		
	Vref (mM)	[CT] (mM)	Biais (%)
LNE 1	3,62	3,74	3,3%
LNE 2	5,96	6,07	1,9%
LNE 3	3,62	3,79	4,8%
LNE 4	5,96	6,05	1,6%
A	3,49	3,35	-3,9%
B	3,47	3,26	-6,1%
C	6,94	6,65	-4,2%
D	4,62	4,79	3,7%
E	4,01	4,15	3,5%
F	6,12	6,23	1,8%
G	2,90	2,92	0,9%

Bias observed on frozen and lyophilized materials can be very different !

Lyophilized materials very often had a lower commutability level compared to Frozen materials, especially those prepared according to NCCLS C37-A

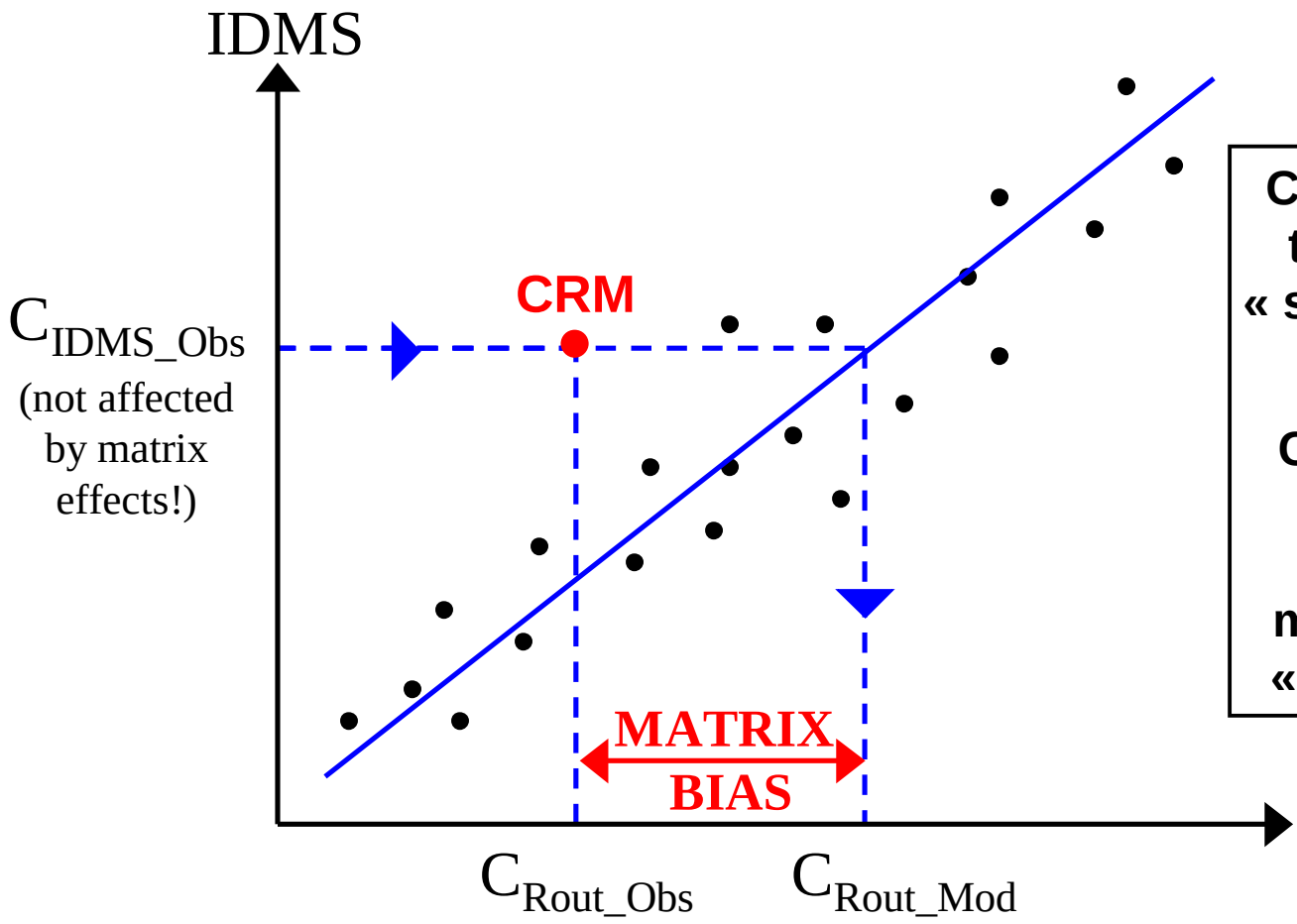
Data analysis

Step 1 : Linear regression à Generalized Least Squares (XLGenLine)



Data analysis

Step 2 : Determination of the matrix bias associated with the CRMs



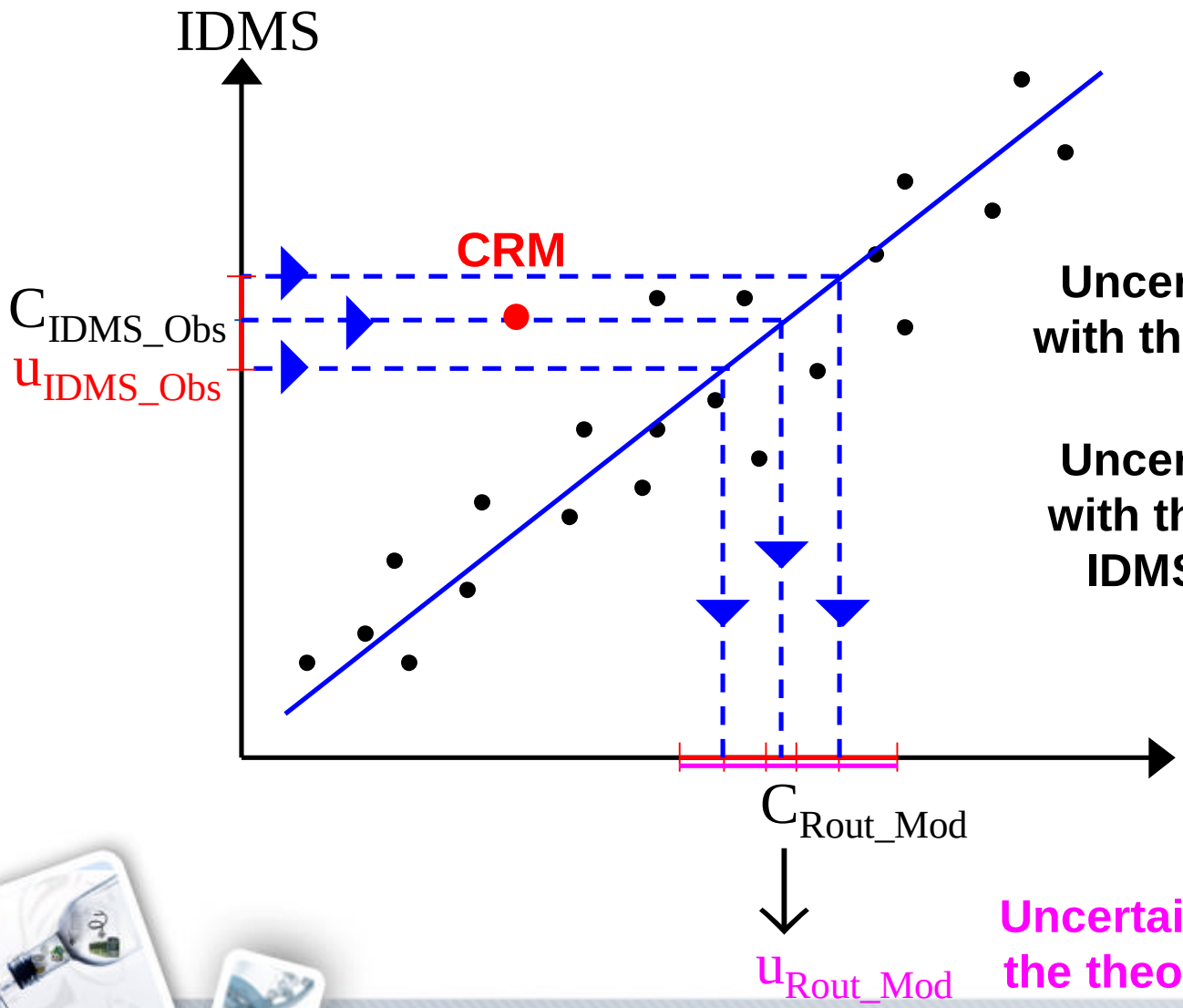
C_{Rout_Mod} is computed from the regression model by « simulating » matrix effects on the IDMS value.

C_{Rout_Mod} is the modelized value that should be measured by the field method if the sample was « perfectly » commutable.

à **What is the maximum allowable matrix bias for a material to be considered commutable?** à **Determination of confidence intervals**

Data analysis

Step 3 : Determination of the acceptance criterion for commutability



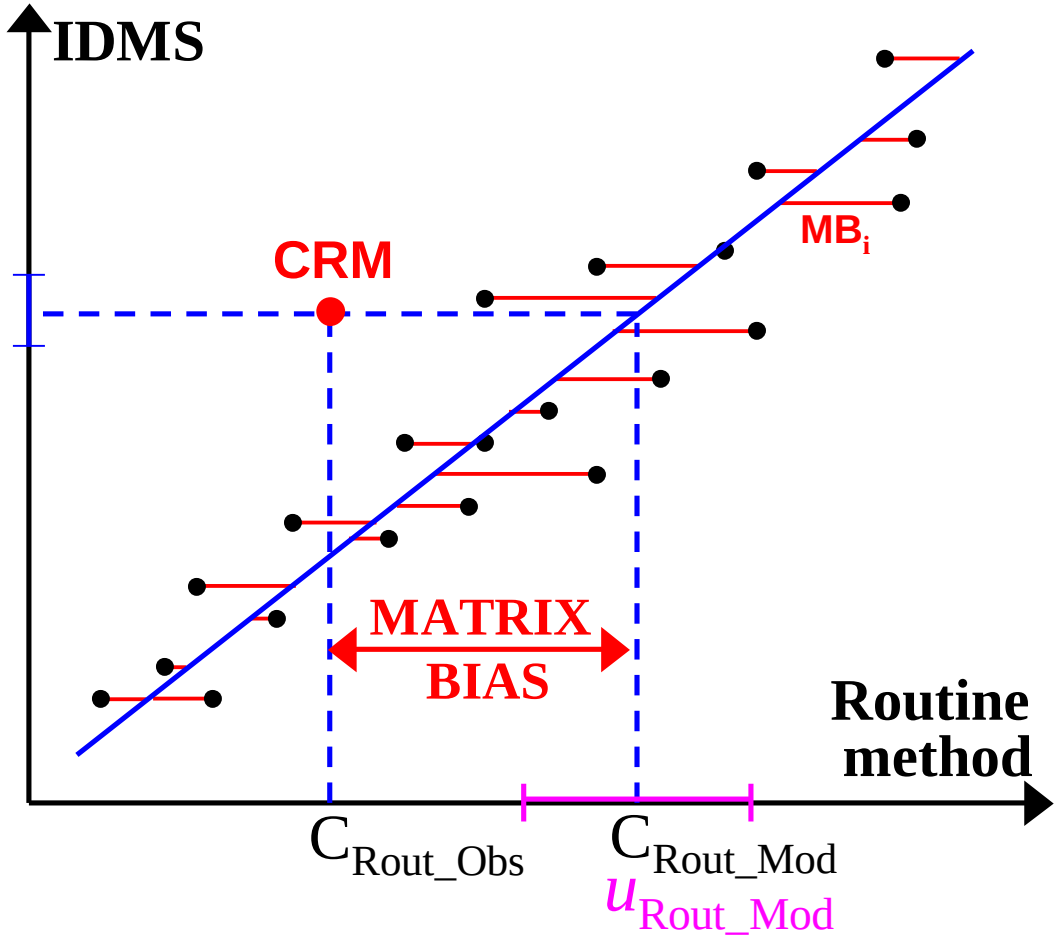
Uncertainty associated with the regression model

Uncertainty associated with the precision of the IDMS reference value

Uncertainty associated with the theoretical routine value

Data analysis

Step 3 : Determination of the acceptance criterion for commutability



Determination of the matrix bias of each INDIVIDUAL native sample and the associated uncertainty :

u_{MBi}

(native sample i)

Uuncertainty associated with the mean matrix bias of the OVERALL SET of native samples

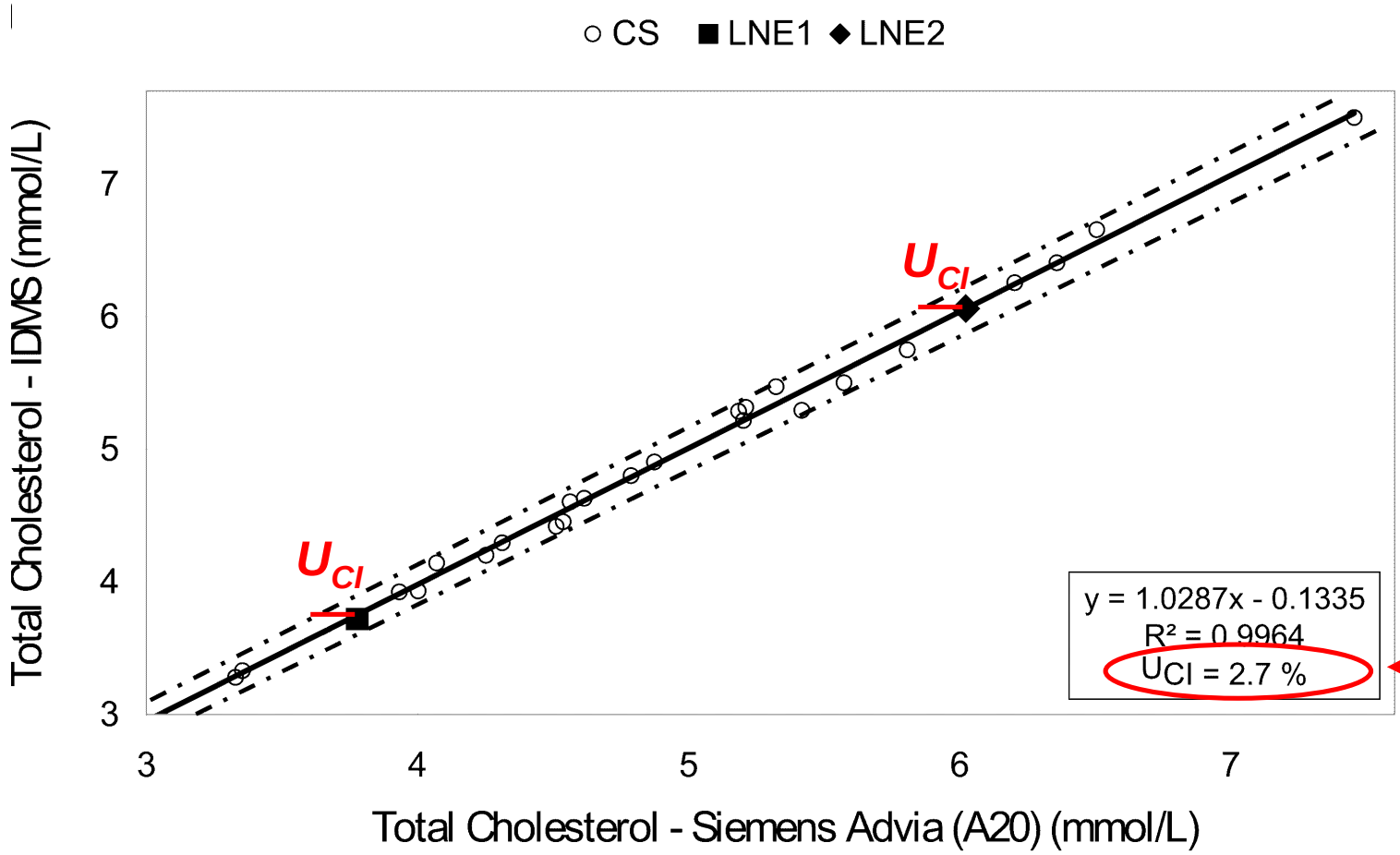
$$u_{\text{matrix bias (of the overall set of native samples)}} = \sqrt{\frac{\sum_{i=1}^{n} u_{MBi}^2}{n}}$$

Expanded uncertainty associated with the modelized value C_{Rout_Mod}

$$U_{CI} = k \times \sqrt{u_{MatrixBias}^2 + u_{Rout\_Mod}^2}$$

« Global » component **« local » component**

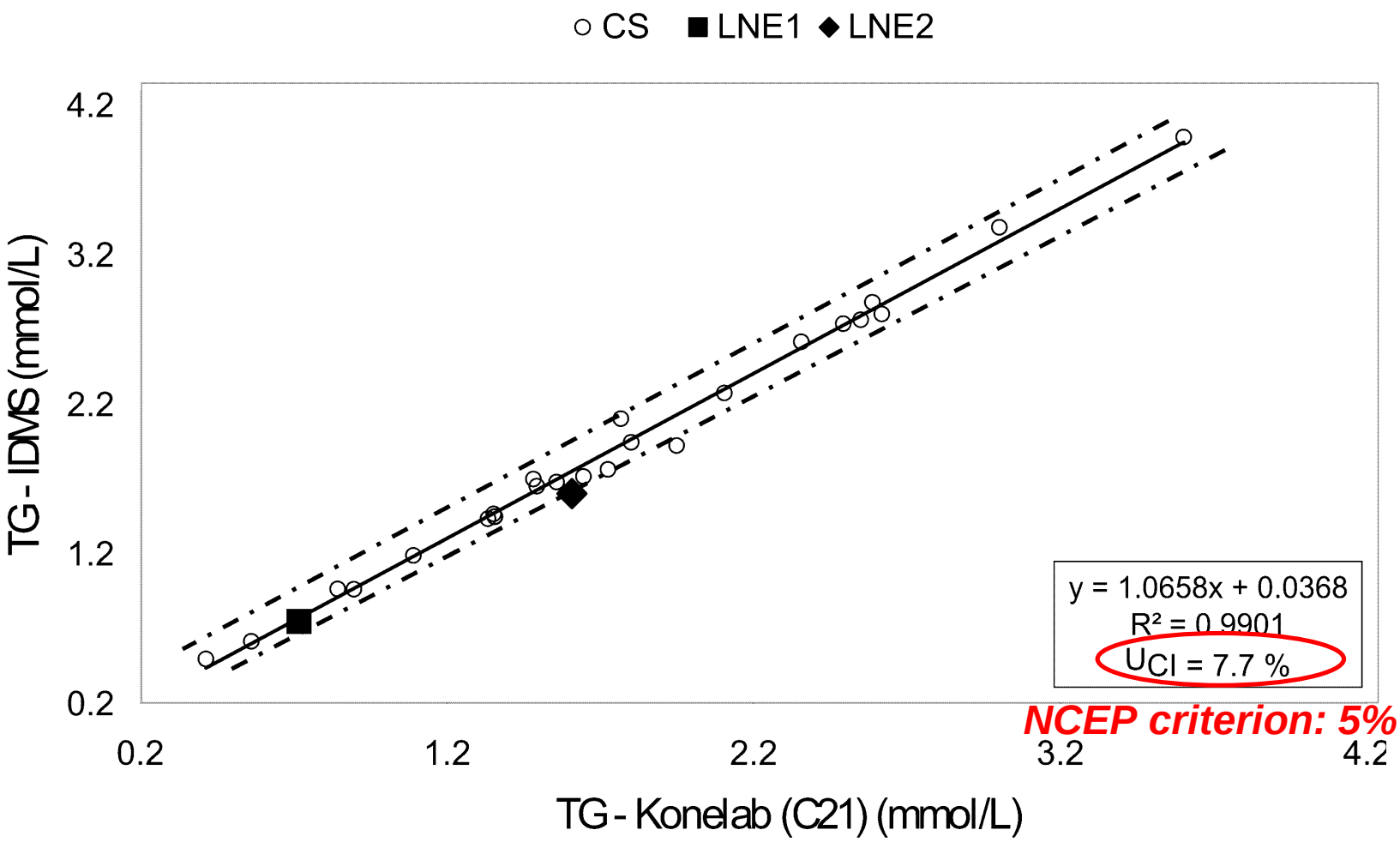
Examples : TCh



Relative U_{CI} normalized to C_{Rout_Mod}

Maximum bias recommended by the NCEP : 3%

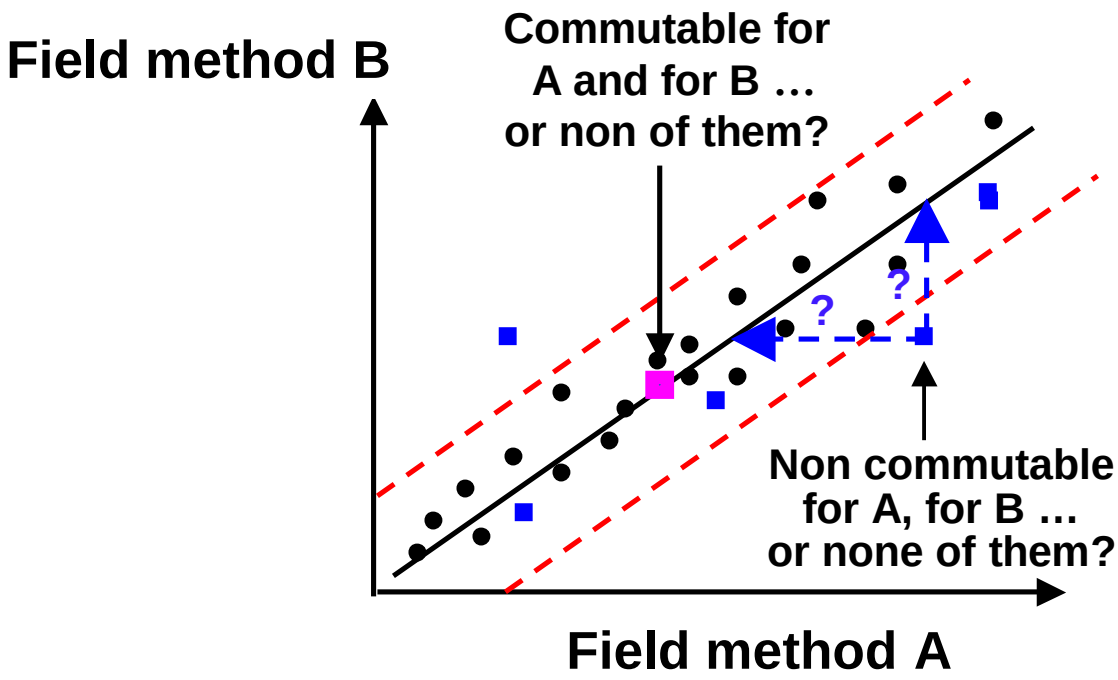
Examples : TG



NCEP criterion: 5%

à acceptance criteria should be defined according to the intended use of materials

The importance of using reference methods



- à **It is highly desirable to analyze native samples with a reference method that is not sensitive to matrix effects, otherwise matrix effects can either compensate or cumulate each other** à **misleading conclusions**
- à **When such methods exist (eg. IDMS) : huge amount of work for a ref lab (400 samples received x 5 parameters = 2000 reference measurements!!)**
- à **When they don't exist** à **pair-wise comparisons between field methods only**

Pair-wise comparisons between field methods

C-HDL

	VA	VD	VZ	KA	3K	SB	W6	YE
VA				A03 B03	A01 B01	A11 B11	A07 B07	
VD				A02 B02	A19 B19	A09 B09		A16 B16
VZ				A04 B04		A08 B08		
ZA					A15 B15			A14 B14
3K								
SB								
W6								
YE								

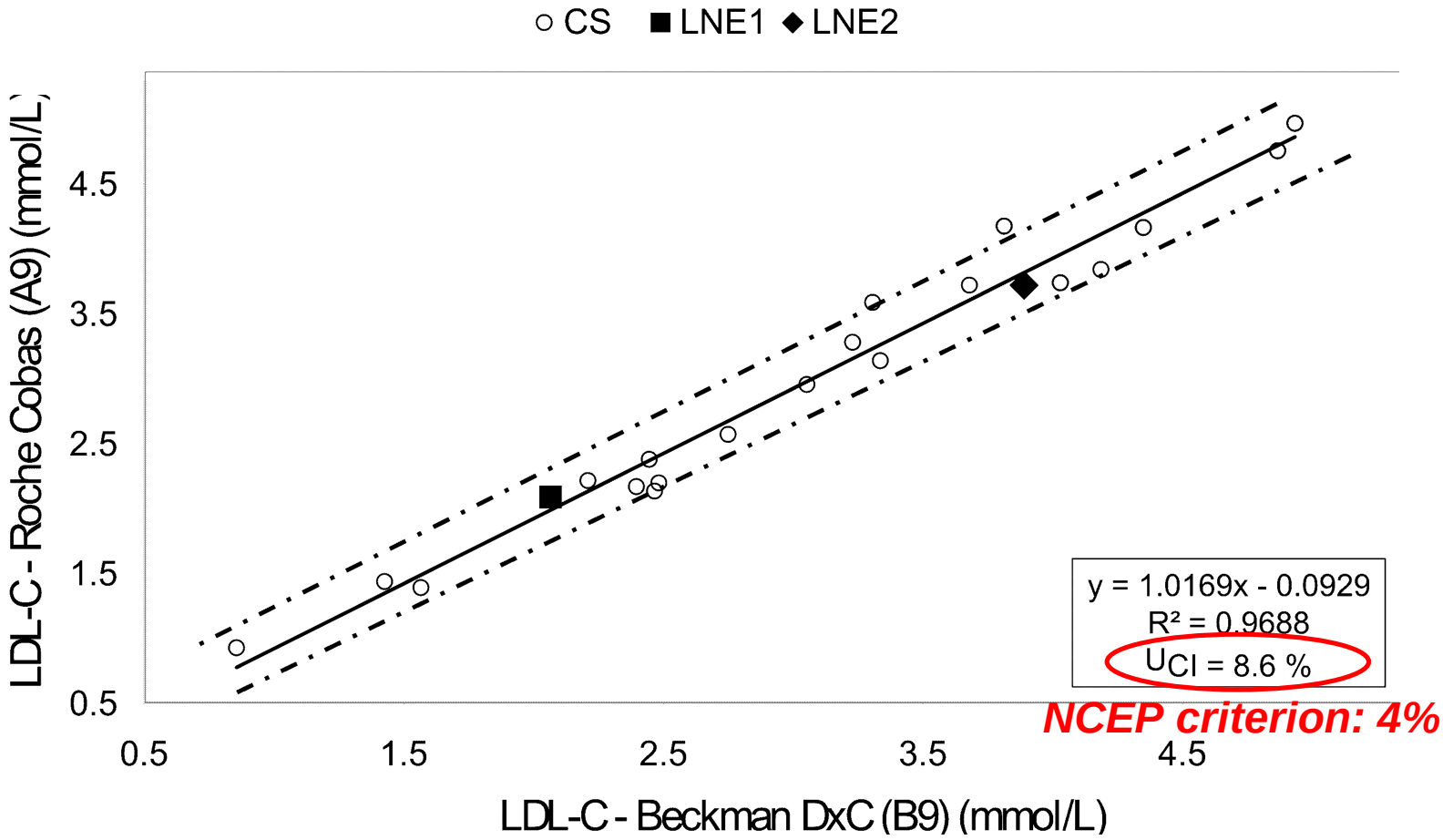
Pair-wise comparisons between field methods only

à **Need to have the highest possible number of methods combinations**

C-LDL

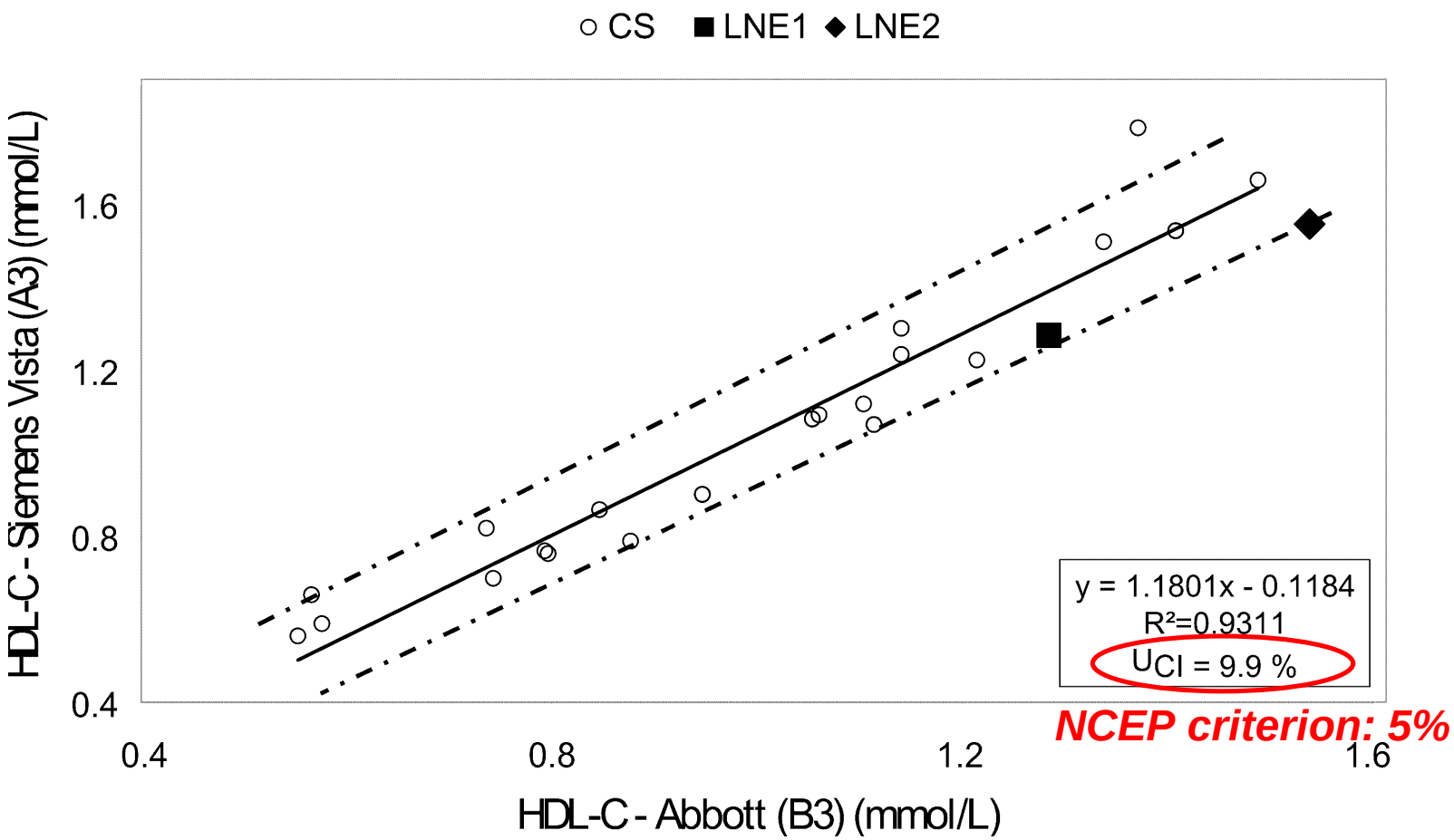
	SD	CX	SZ	SH	CX	SA	CX	YK	CX	SB	CX	SO	CX	CX
SD						A03 B03	A03 B03		A01 B01					
CX						A03 B03	A03 B03		A01 B01	A11 B11	A07 B07 + A11 B11			
SZ							A02 B02							
SH							A04 B04			A09 B09	A09 B09	A08 B08	A08 B08	
CX							A04 B04 + A02 B02	A19 B19	A19 B19	A09 B09	A09 B09	A08 B08	A08 B08	A16 B16
SA														
CX									A15 B15					A14 B14
YK														
CX														
SB														
CX														
SO														
CX														
CX														

Examples : LDL-C



NCEP criterion: 4%

Examples : HDL-C



NCEP criterion: 5%

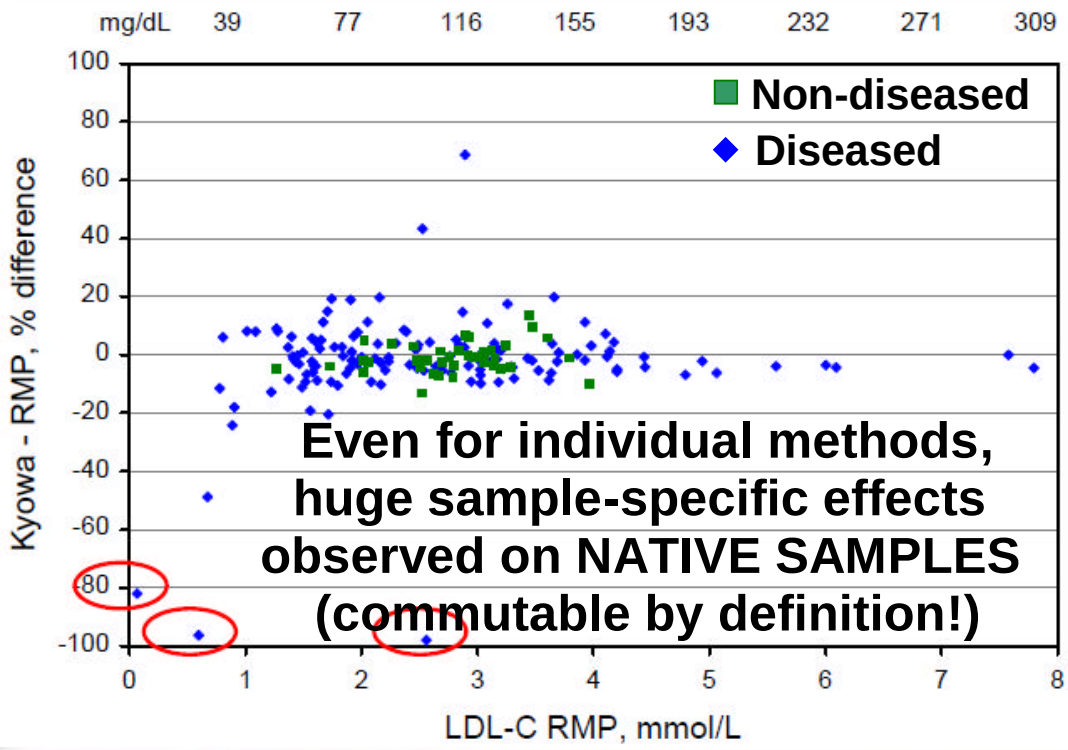
- **Large confidence intervals à materials found commutable too easely?**
- **Maybe but more stringent acceptance criteria would result in a high number of native samples to be found non-commutable !!**

Selecting representative clinical samples

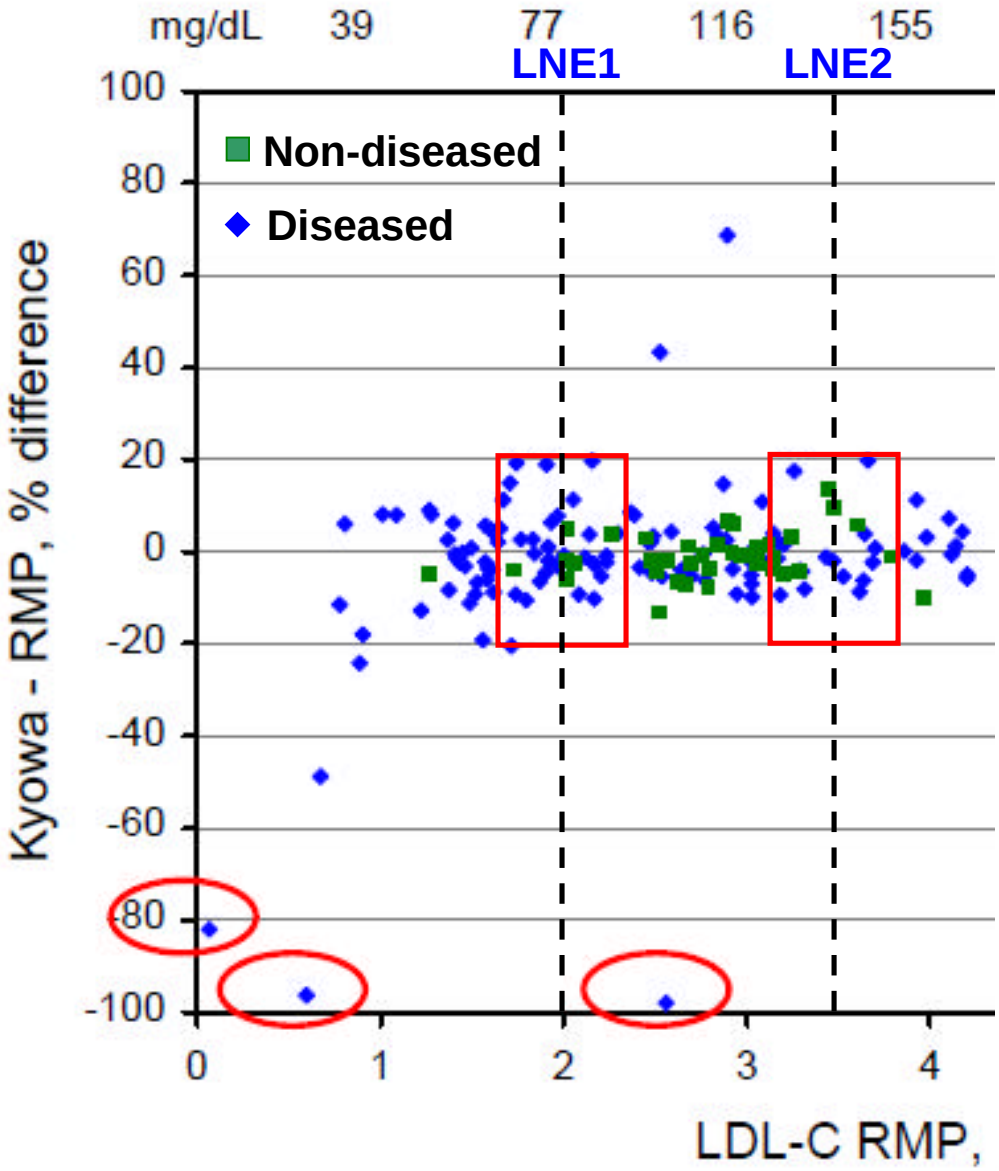
Clinical Chemistry 56:6
977–986 (2010)

Seven Direct Methods for Measuring HDL and LDL Cholesterol Compared with Ultracentrifugation Reference Measurement Procedures

W. Greg Miller,[1*] Gary L. Myers,[2] Ikunosuke Sakurabayashi,[3] Lorin M. Bachmann,[1] Samuel P. Caudill,[2] Andrzej Dziekonski,[1] Selvin Edwards,[2] Mary M. Kimberly,[2] William J. Korzun,[1] Elizabeth T. Leary,[4] Katsuyuki Nakajima,[5] Masakazu Nakamura,[6] Göran Nilsson,[7] Robert D. Shamburek,[8] George W. Vetrovec,[1] G. Russell Warnick,[9] and Alan T. Remaley[8]



Selecting representative clinical samples



- **Biais measured on different native samples can vary a lot!**
  - à **need to estimate trueness with more than one PT sample!**
- **These results highlight lack of specificity of methods and/or a problem of standardization :**

All methods don't measure the same thing!

- à **Need for advanced analytical techniques to better understand what methods really measure!**

EMRP Project

Thank you for your attention

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