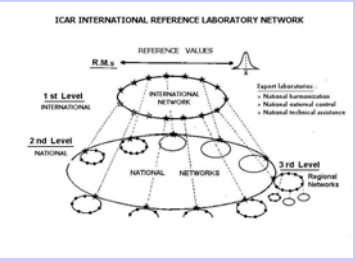


Interlaboratory reference system & centralised calibration

Pre-requisites & standard optimum procedures

Olivier Leray, Cecalait, france

Introduction

Objectives for ICAR

> Harmonise, optimise accuracy of reference values used for calibration ⇒ reduce overall uncertainty of routine results

> Provide true values to analytical sites where reference methods impossible (e.g. inaccurate ref method ; on-farm analysis)

> Reduce analytical cost by sharing and amortising calibration costs on numerous analyses.

Introduction

Reference system and centralised calibration

> System allowing

- to establish a unique reference valid for a community of laboratories
- to transfer consensus reference values to laboratories to calibrate routine methods
- to assess functioning of the system

> refer to a general analytical system chosen for a prior defined purpose (i.e. milk recording)

> part of a strategy to achieve the objectives of organised users, thus resulting from a collective choice

Pre-requisites of centralised calibration

1- Geographic area : ⇒ No / limited matrix effects *Overall accuracy with vs without centralised calibration ; matrix effects* ⇒ *choice*

2 - Laboratory group : ⇒ homonegeity for methods, criterion expression, units

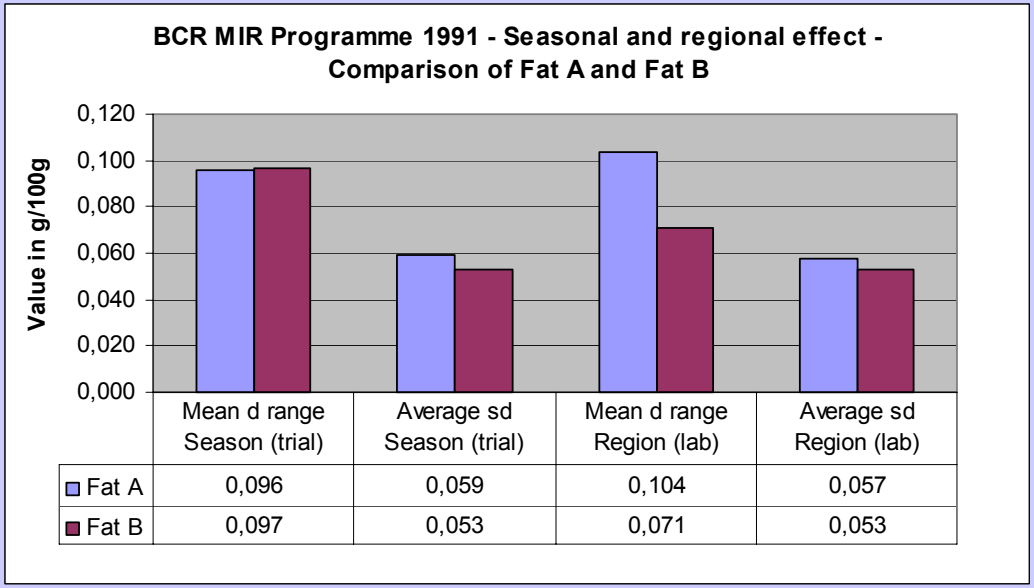
3 - Sample preservation : ⇒ Adequate to required shelf life

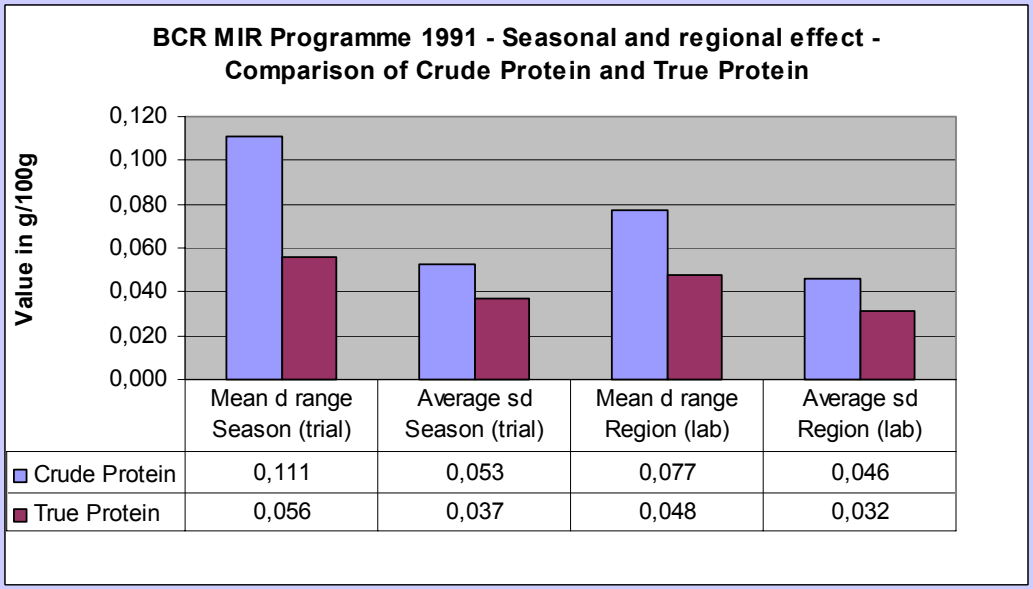
4 - Logistic : Sample transport facilities ⇒ safe, in time

Mid infra red spectroscopy and matrix effects on classical wavelengths

Components	Wavelength λ (µm)	Interferents corrected	Interferents uncorrected	Influencing factors	Origins
Fat	5,7	(Protein)		FA Molecular weight	Diet, feeding (season, region); species (metabolism)
		(Lactose)		Ester linkage breaking (lipolysis)	Sample mishandling ; stage of lactation ; species
Fat	3,5	Protein Lactose			
			c=c	Unsaturated fatty acids (UFA)	Diet, feeding (season, region)
			FFA		Sample mishandling ; stage of lactation ; species
Protein	6,5	Fat Lactose	FFA carboxylic acids (citrate, lactate)		Sample mishandling ; stage of lactation ; species Diet, feeding (season, region); species (metabolism)
				NPN in CP calibration	Diet, feeding (season, region); species (metabolism)

FT-MIR Full Spectrum ?





BCR MIR Prog. 1991:

15 European countries (labs)

8 trials on 1 year

2 bulk milks/trial/lab

Protein expression Crude vs True Protein

Variation	Concentration	Range	Trials
Seasonal	0,14 - 0,22 0,14 - 0,24	0,08 0,10	INRA (BCR1992) Cecalait (1992-1996)
Within region	-	0,05	Cecalait (1996)
Between regions (FR)	0,14 - 0,22	0,08	Cecalait (1996)
Between CE countries	0,17 - 0,21	0,04	INRA (BCR1992)

ICAR strategy : Means & tools

> Develop ICAR guidelines on :

- organising interlaboratory proficiency studies (PTs)
- organising centralised calibration (CC)

> Provide / develop ICAR services :

- international proficiency studies (IPTs)
- international reference materials (IRMs)

to be relaied towards national levels

⇒ promote national PTs and CC

About ICAR Guidelines for Interlaboratory Proficiency Study

- **For both reference and alternative methods**

- **Consistency with ISO 13528 and IUPAC protocol**

- **Consistency with calibration issue (ISO 8196) :**
  > *samples* $N_S \geq 9$
  > *concentrations = normal calibration ranges in milk*
  > *levels* $N_L \geq 3$
  > *design : arrangement for optimised assessment (ISO 9622)*

- **Statistical evaluation :** Usual performance scores
  + instrument fitting assessment
  (slope, linearity, interactions)

About ICAR Guidelines for Centralised Calibration

1- Evaluation for choice of central calibration :

a- Picture of current situation ⇒ PTs (ref / routine)

b- Evaluation of overall accuracy ⇒ region & season effects

2 - Characteristics of calibration RMs :

⇒ quality, safety, preservation, shelf life, fit-to-purpose

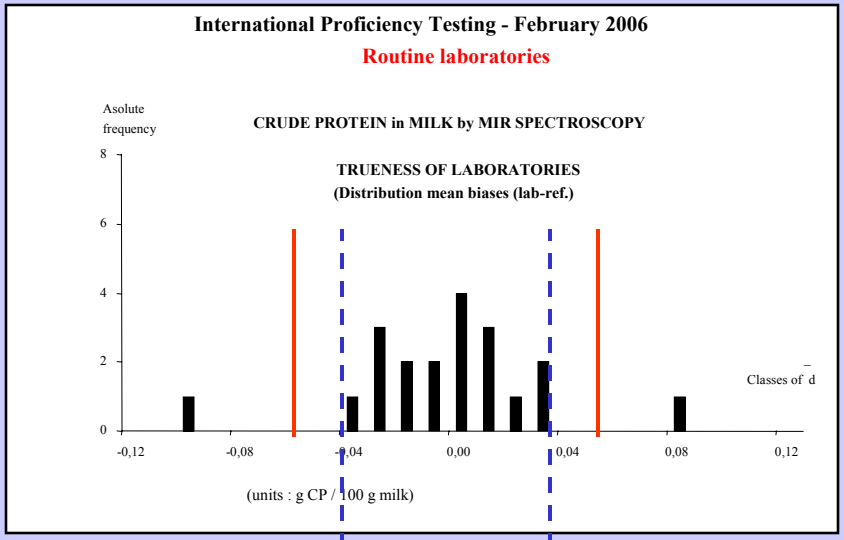
3 - Assign reference values ⇒ laboratories, organisation

4 - Calibration ⇒ pre-calibration, local correction

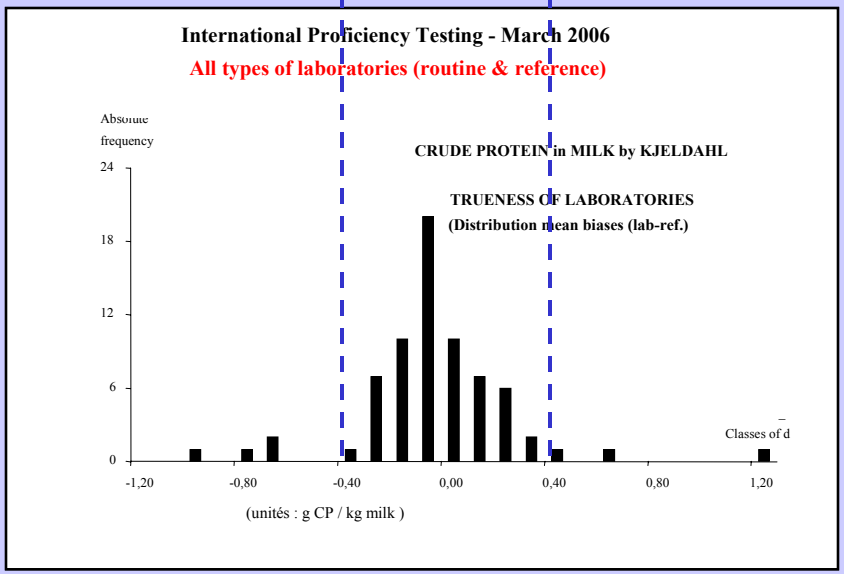
⇒ external = PTs , internal = ISO 8196

1a - Evaluation of current situation through PTs

Routine



Reference



Principle

(Quarterly) comparison thr. PTs:

- simultaneously
- same q samples (n repl.)
- same p laboratories
- local calibrations

For collective purpose:

1- $sd_{rout} \approx d_{ref}$ => OK

2- $sd_{ref} < sd_{rout} < \sqrt{2}.sd_{ref}$

=> lab effect acceptable

3- $\sqrt{2}.sd_{ref} < sd_{rout}$ => discrepancy in overall accuracy

Decision : sd_{rout} acceptable / not ?

1b - Evaluation of overall accuracy in centralised calibration

Experiment (same method used in laboratories)

1- Throughout a whole cycle of milk production (8-12 months)

2- Coverage of regions / labs involved in centralised calibration

3- One instrument in the evaluating laboratory

1- Analyse : representative test samples of different collect areas (labs) by the routine methods in a same calibration and the reference methods.

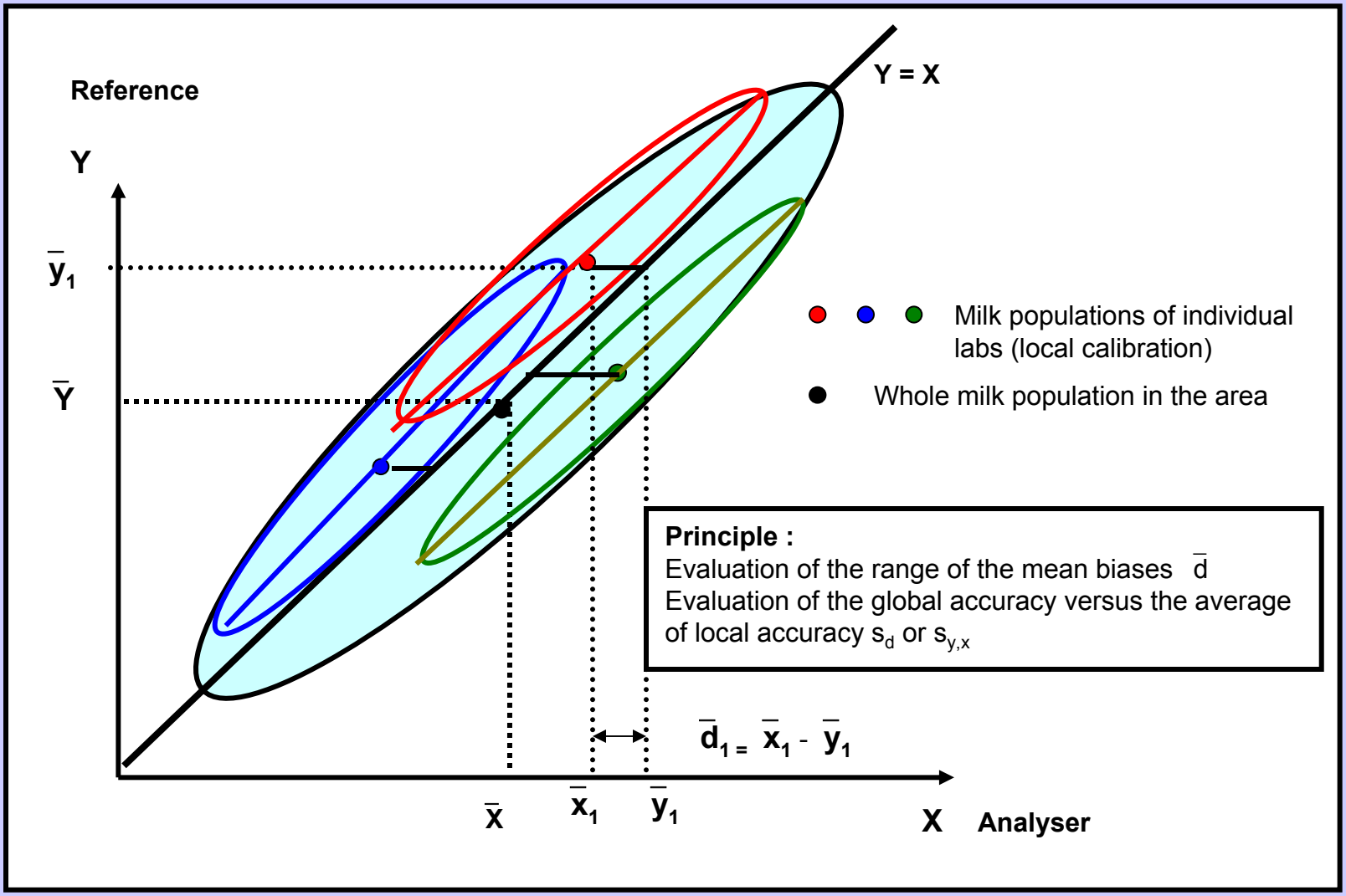
2- Calculation:

- differences and mean differences in a unique calibration for all (periods x labs)
- Individual one-way ANOVA's per season and region : Effect of regions and season
- Two-ways ANOVA (region x season) : Crossed effect (interaction)

3- Evaluation : - ranges of variation of calibration bias between labs and between periods
- overall accuracy standard deviation and per region and season

4- Decision : by reference to maximum acceptable limits (ICAR guidelines)

Principle of the evaluation of the regional effect and of the possible accuracy resulting of a centralised calibration



From draft guidelines :

Table 4 - Table of mean and standard deviation of differences with the reference method

Region	Period j				
i	**1**	**…**	**3**	**…**	**q**
1					
2			$\bar{d}_{ij}$ sd_{ij}		
…					
p					$\bar{d}_{pq}$ sd_{pq}

Period effect per region						
$\bar{d}$	$s_{\bar{d}}$	**sw**	**sd**	F_{obs}	**LSD**	**LSB**
$\bar{d}_{i.}$	$s_{\bar{d}\,i.}$	$sw_{i.}$	$sd_{i.}$	F_{obs}	$LSD_{i.}$	$LSB_{i.}$
$\bar{d}_{p.}$	$s_{\bar{d}\,p.}$	$sw_{p.}$	$sd_{p.}$	$F_{p.}$	$LSD_{p.}$	$LSB_{p.}$

Region effect per period					
$\bar{d}$			$\bar{d}_{.j}$		$\bar{d}_{.q}$
$s_{\bar{d}}$			$s_{\bar{d}\,.j}$		
sw			$sw_{.j}$		$sw_{.q}$
sd			$sd_{.j}$		$sd_{.q}$
F			$F_{.j}$		$F_{.q}$
LSD			$LSD_{.j}$		$LSD_{.q}$
LSB			$LSB_{.j}$		$LSB_{.q}$

Global analysis						
$\bar{d}_{...}$	$s_{O\,\bar{d}}$	$sw_{...}$	$sd_{..}$	F_P	$LSD_{..}$	$LSB_{..}$
$s_{P\,\bar{d}}$						
$sw_{...}$						
$sd_{..}$			$sd_{..}$			
F_O						
$LSD_{..}$					$LSD_{..}$	
$LSB_{..}$						$LSB_{..}$

2 - Characteristics of calibration RMs

1 - Physico-chemical quality of milk :

> Recent milking (day) : *bacteriological quality !*

> Milking : only little air incorporation in milk *lipolysis !*

> No thermal / physical shocks : *churning, oiling-off !*

⇒ commingle selected herd milks *better than bulk milk of dairies*

2 - Characteristics of calibration RMs

2 - Sample preparation :

> Milk handling : Gentle at sampling / preparation / splitting in vials

> Storage : 4°C with preservative (if work not on the day)
No light ; no (little) air in contact

> Splitting : - Regular mixing with no air incorporation
- Vials well filled (small headspace => big air bubble)

> Checks : Homogeneity / stability (ISO 13528)

2 - Characteristics of calibration RMs

3 - Preservation, container & storage :

> Chemicals : - safety to persons & environment
- no interference with reference methods

⇒ *against bacteria (bronopol), moulds (natamycin)*

> Physical option : deep freezing at -80°C *(lower vial filling)*

> Containers & caps : Unbreakable, no leakage

⇒ *PPHD, screw caps, airtight joints*

> Shelf life : 4°C : 6 weeks
-20°C : several months

2 - Characteristics of calibration RMs

4 - Fit for the purpose of instrument fitting & calibration :

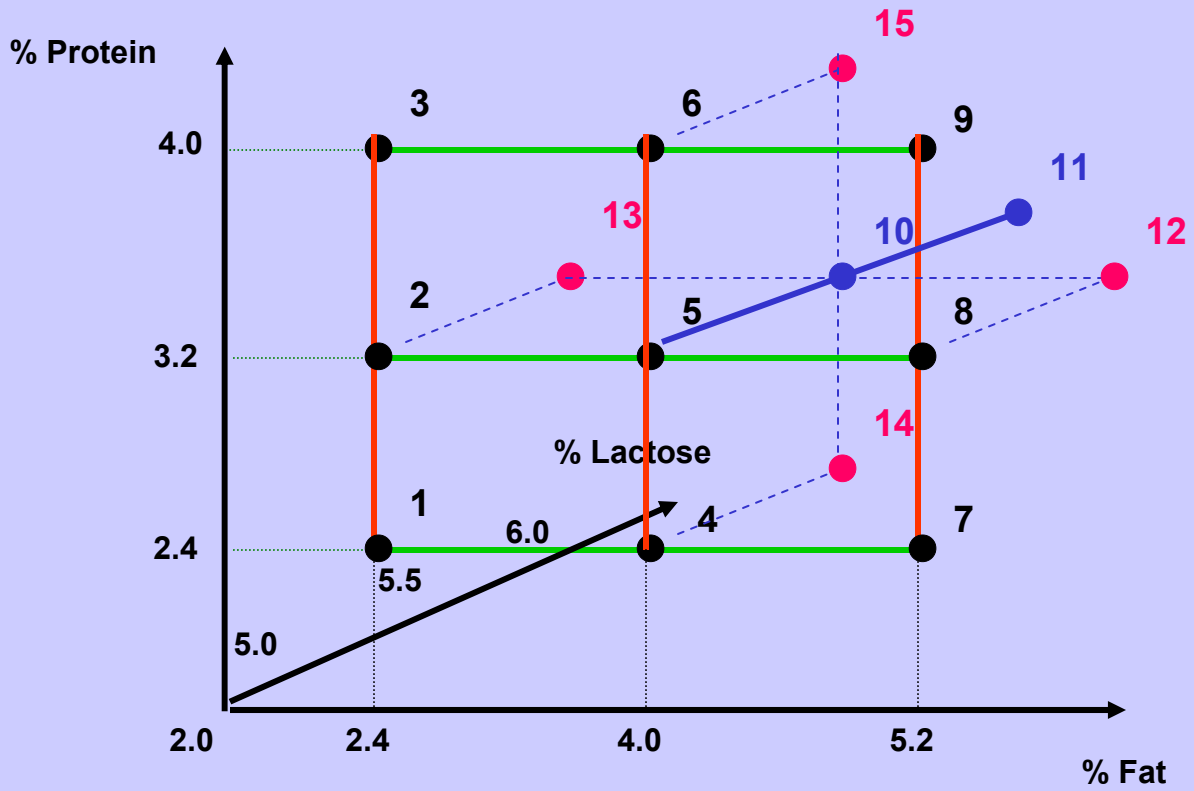
> Number : $q \geq 9$ (ISO 8196)

> Concentration : Coverage of usual ranges

> Sample set design : Maximum contribution to slope, linearity, interaction evaluation

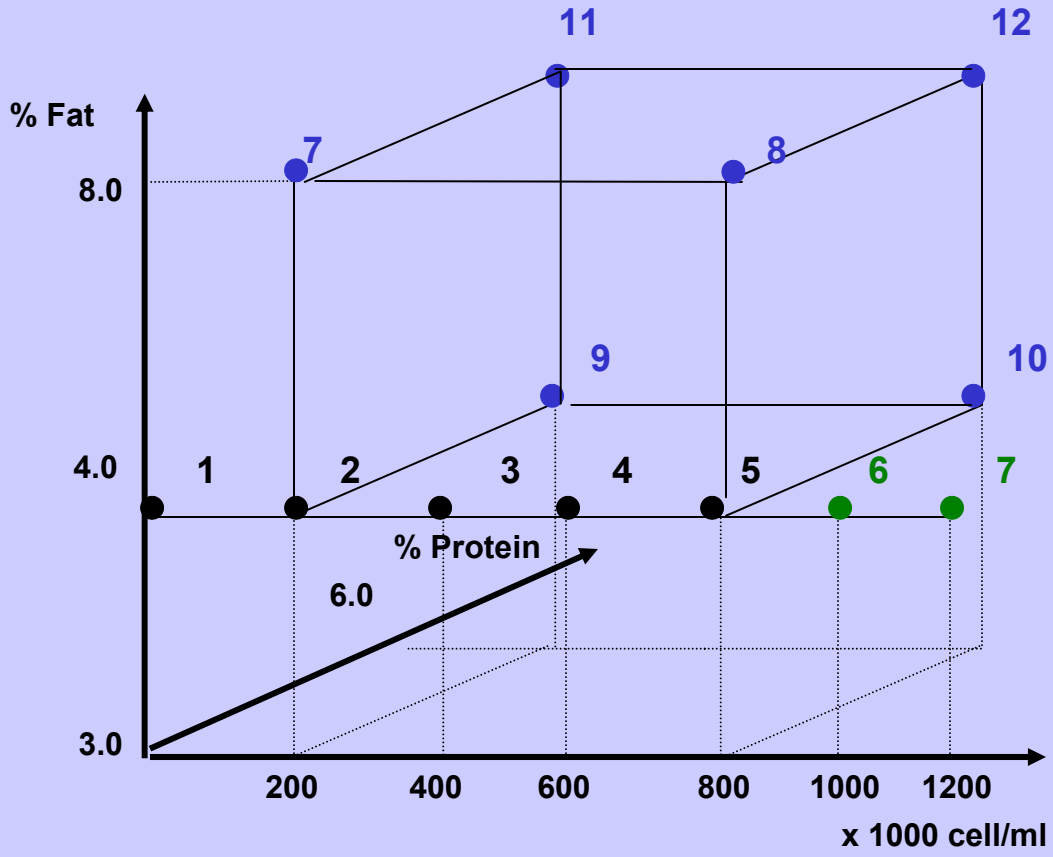
$\Rightarrow$ *recombined milk samples in orthogonal experimental design*

Example : Experimental design for MIR calibration (recombined samples)



O. Leray, 1988, 1990, 1998, IDF 141

Example : Experimental design for SCC calibration (recombined samples)



- **calibration, linearity**
- **interference** *sheep, goat & buffalo*
- **complement**

Cecalait, CE Programme FAIR, 1997-1999

3 - Assign reference values

1- Routine methods with no matrix effect : (e.g. SCC)

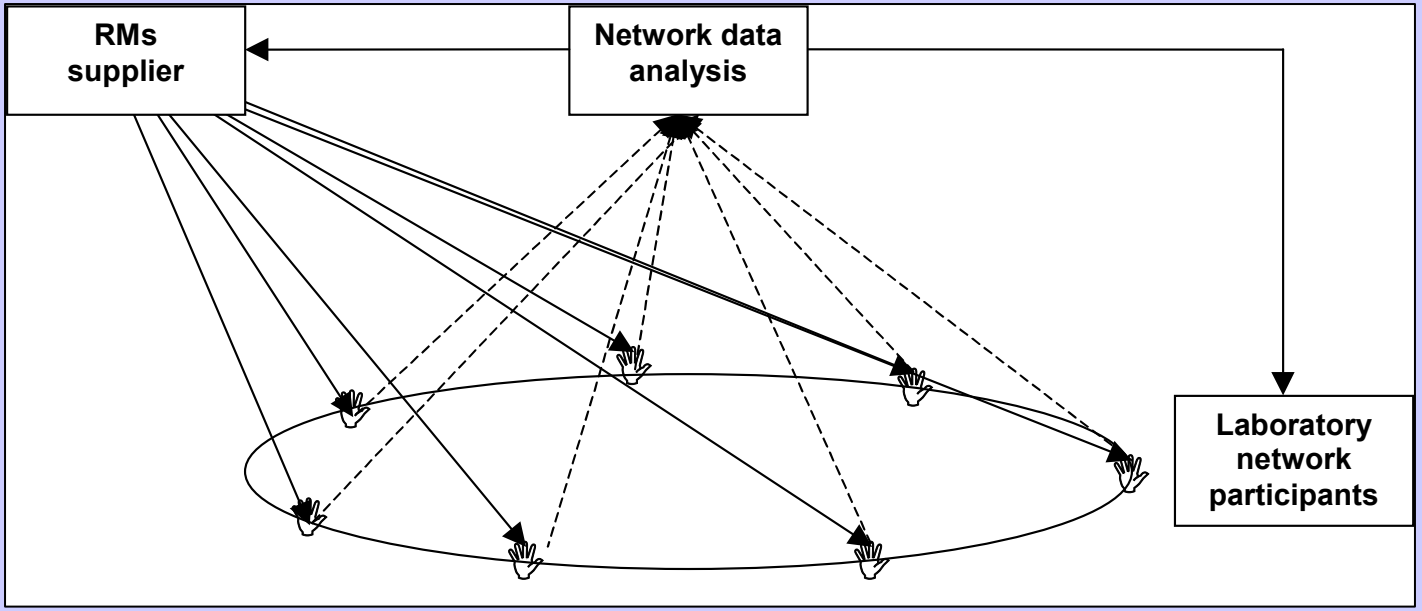
> Method : Reference methods (IDF/ISO)

> Laboratories :

- Interlab study : group members / larger group / selected expert labs

- CRMs / IRMs : Reference laboratory relaying international gold standards (master analyser)

Central RM system for method with no matrix effect



General model : Numerous laboratories and samples ; robust reference

3 - Assign reference values

2 - Routine methods with matrix effect : (e.g. MIR)

> By the organiser laboratory

- Reference method values
- Values calculated from accurate mixing ratios

⇒ *Region/lab bias correction may be needed (milk not representative)*

Where regional effect acceptable (no bias correction) :

- **Centring** on regional average of instrumental responses

⇒ *Minimize overall calibration error*

3 - Assign reference values

> Centring of reference values :

- Milk sample(s) representative of each lab area and calibration samples analysed simultaneously in reference and routine :

1- Interlab study : by laboratories ⇒ different routine methods

2- In-house study : by the organiser ⇒ same routine method

⇒ *master instrument*

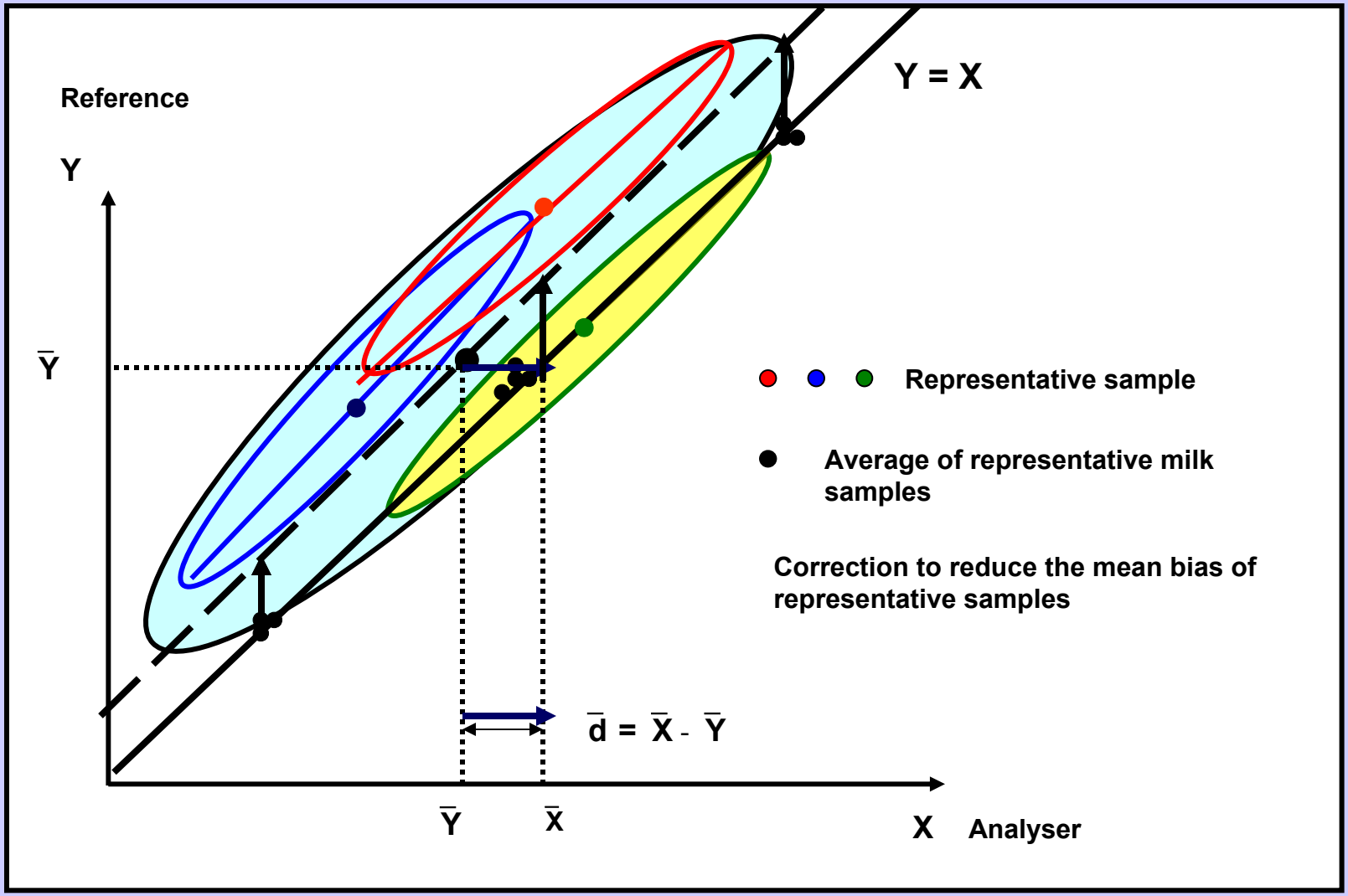
- Biases on reference (1 or 2) corrected by concomitant CRM/PT

- Align labs results in one medium calibration giving values X_L

- Calculate the averages of all lab samples $\bar{Y}_L$ **(ref)** & $\bar{X}_L$ **(rout)**

$$Ref_C = Ref_R \cdot (\bar{Y}_L / \bar{X}_L) \quad \text{or} \quad Ref_C = Ref_R - (\bar{X}_L - \bar{Y}_L)$$

Centring theoretical values for centralised calibration



3 - Assign reference values

> Individual region/lab correction :

- From results with representative sample(s) of the region and reference optimised thr. simultaneous PT / CRM analyses

- By the organiser: identified laboratory group of labs Li

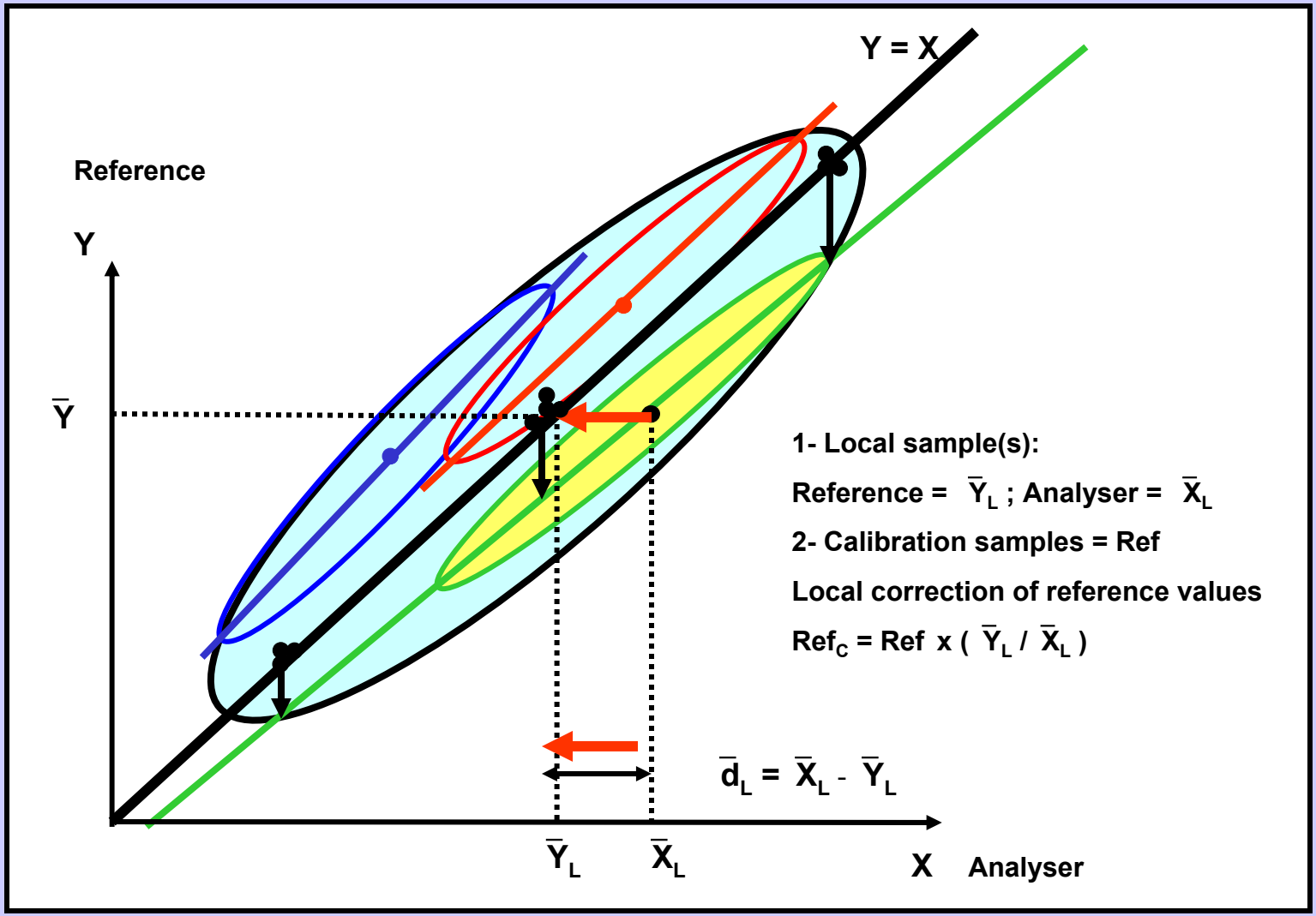
  ⇒ possible individual cal monitoring thr. internet

- By the laboratory (Li): open system with pre-calibration

⇒ Final correction:

$$Ref_{Ci} = Ref_R \cdot (\bar{Y}_{Li} / \bar{X}_{Li}) \quad \text{or} \quad Ref_{Ci} = Ref_R - (\bar{X}_{Li} - \bar{Y}_{Li})$$

Local bias correction to reduce existing region effect



4 - Calibration

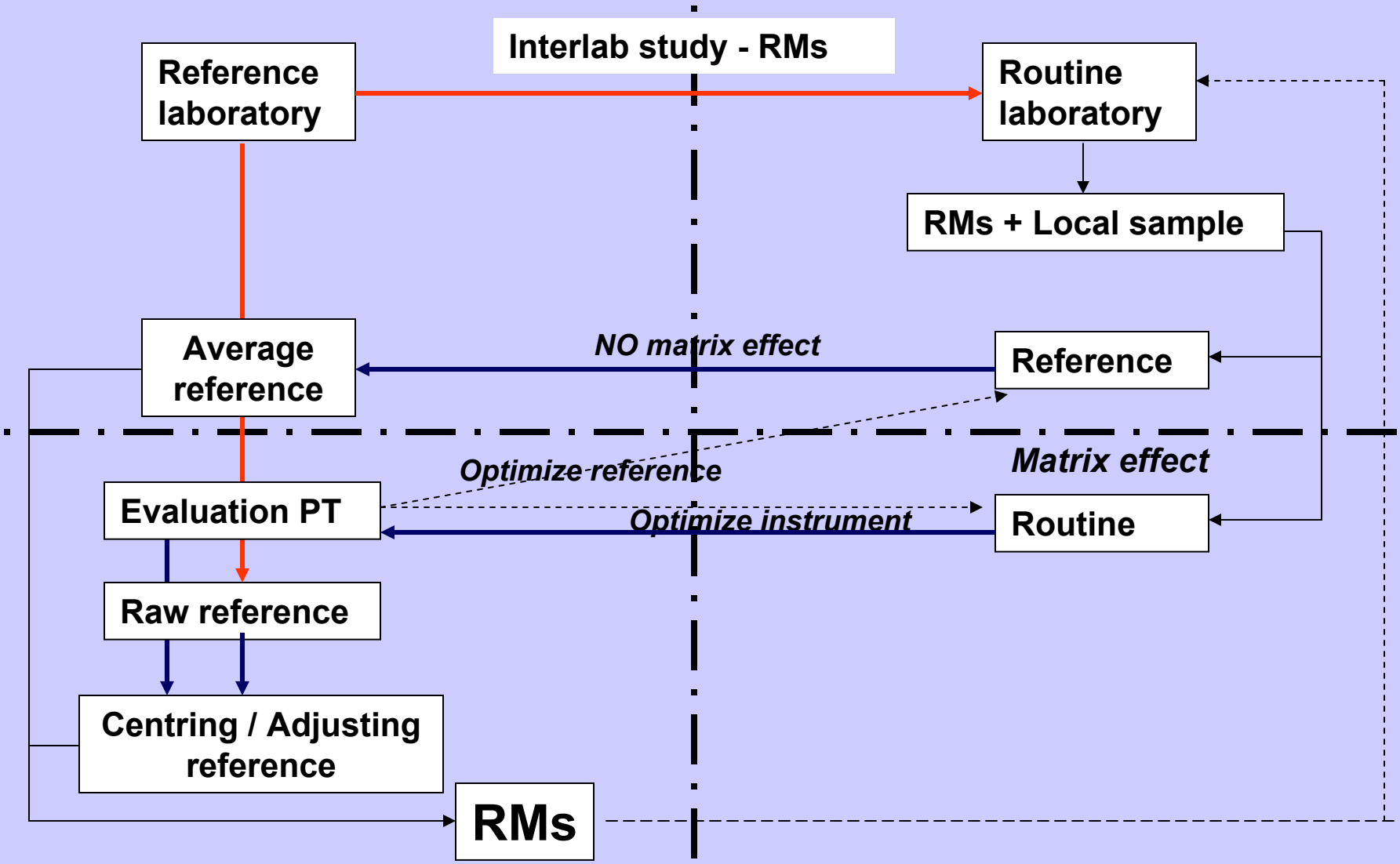
According to IDF 128 /ISO 8196

1 - Check / optimize instrument fittings

2- Calibration / pre-calibration

3 - Final calibration and assign values for control samples

Integrated system



Conclusion

- Appropriate tools and procedures for the application of centralised calibration already exist

- Suitable optimum methodologies and procedures are being developed as to be described in ICAR Guidelines

- Centralised calibration is a logical step in laboratory anchorage to international true values via reference laboratories.

- Centralised calibration can provide ease and security for calibration to laboratories and can be the adequate way to calibrate on-farm milk analysers.

Thank You for your attention !