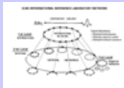

Reference and calibration system for routine milk testing

Advantages & Disadvantages - Choice criteria

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Introduction

Reference and calibration systems

⇒ 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 organied users, thus resulting from a collective choice.

Objectives

⇒ to optimise the accuracy of results (or lower the related uncertainty)

with providing sufficient confidence in the quality of results

and with an acceptable balance between quality & cost

The error of measurement includes :

⇒ **the precision error of the routine method :**
- repeatability & within day reproducibility (short term stability)
- under control
- cannot be avoided nor reduced in routine testing

⇒ **the precision error of the reference method :** $s_R^2 = s_L^2 + s_r^2$
- repeatability : negligible as reduced by sample and replicate numbers
- reproducibility : Possible systematic error of the lab allowed by the method and normally distributed according to s_L

⇒ **the error of calibration :**
- statistical error of adjustment : can be improved with appropriate samples made to maximize the correlation coefficient
- error of sample representativeness resulting of matrix effects

Analytical systems available for milk recording

	Reference	+	Calibration
INDIVIDUAL	Possible systematic bias $\bar{d}$ of the lab from the standard precision of the method: $s_{\bar{d}}^2 \approx s_L^2$ with : $s_R^2 = s_L^2 + s_r^2$	(2)	**Local calibration :** - High cost (sample collect ; reference analysis) - Additional competence - No bias related to matrix effects (representativeness)
	↕ (1)	(4)	
COLLECTIVE	No/negligible error $\bar{d}$ in estimating true values : $s_{\bar{d}}^2 \approx s_L^2/p$ with : p labs	(3) (5)	**Centralised calibration :** - Low cost (purchase RM samples ; no reference method by labs) - Matrix effects (to be limited) - Competence for testing

(1): PT for AQA ; (2): Isolated lab ; (3): Reference lab ; (4): RMs ; (5): Reference network

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) (Lactose)		FA Molecular Weight; Ester linkage breaking (lipolysis)	Diet, feeding (season, region); species (metabolism); Sample mishandling ; stage of lactation ; species
Fat	3,5	Protein Lactose	c=c; FFA	Unsaturated fatty acids (UFA)	Diet, feeding (season, region); Sample mishandling ; stage of lactation ; species
Protein	6,5	Fat Lactose	FFA; carboxylic acids (citrate, lactate)	NPN in CP calibration	Sample mishandling ; stage of lactation ; species; Diet, feeding (season, region); species (metabolism); Diet, feeding (season, region); species (metabolism)

FT-MIR Full Spectrum ?

Elements for choice

Choice of appropriate methods

Wavelengths : **minimise bias laboratory spreading within the region or country, thus no or only little influence of the milk matrix variation**

Ex: Fat A < Fat B < Fat by FT-MIR Full Spectrum
CP 6,5 µm < CP by FT-MIR Full Spectrum
TP 6,5 µm < TP by FT-MIR Full Spectrum
CP by FT-MIR Full Spectrum ≈ TP by FT-MIR Full Spectrum

Expression units : **routine methods and reference methods to take into account same components in the measurement principle**

Ex: Mass of component : Fat A < Fat B
NPN : Crude Protein 6,5 µm < True Protein 6,5 µm

Elements for choice

1°) Experimental evaluation

Over a one year period and on the whole region :

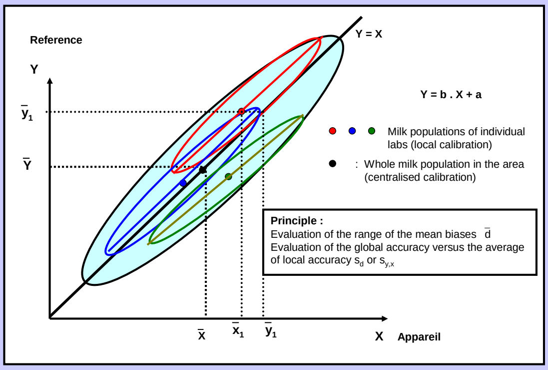
1- Analyse : by a single laboratory representative test samples of different collect areas (labs) by the routine methods with unchanged calibration and the reference methods.

2- Evaluation : of ranges of variation of theoretical calibration bias between labs and between periods

3- Decision : by reference to maximum acceptable range of calibration bias (fit for purpose).

Examples : BCR MIR Programme 1991 within Europe ; Experiments in France (1981-1985).

Example: Evaluation of the regional effect and of the possible accuracy resulting of a centralised calibration

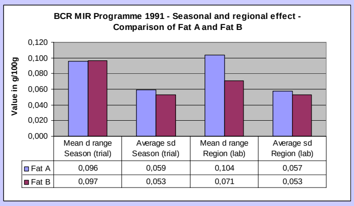


Geographical and seasonal effect on mid infrared fat and protein determination in France:

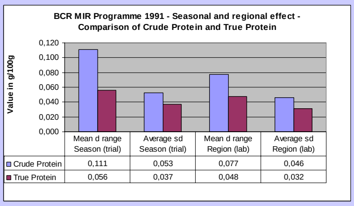
Samples of 8 regions of France with various geographical/seasonal situations analysed at the same time in reference and infra red and calibration (same instrument) optimised on the whole of data for each season to measure local effects (ANOVA).

Measurand (g/100g)	Season	Range of $\bar{d}$	Total sd	Region sd	F test
Fat 5,7 µm	Nov. 1981	0,102	0,082	0,077	3,12 (**)
	Feb. 1984	0,042	0,043	0,043	1,20 (NS)
	June 1985	0,086	0,051	0,044	6,80 (***)
Fat 3,5 µm	Nov. 1981	0,063	0,052	0,047	4,01 (***)
	Feb. 1984	0,017	0,027	0,027	0,90 (NS)
	June 1985	0,031	0,031	0,030	1,90 (NS)
True protein 6,5 µm	Nov. 1981	0,018	0,023	0,023	0,74 (NS)
	Feb. 1984	0,019	0,028	0,029	0,85 (NS)
	June 1985	0,022	0,019	0,018	2,34 (NS)

Units: g/100g O. Leray, Le Lait, 69, 1989



	Mean d range Season (trial)	Average sd Season (trial)	Mean d range Region (lab)	Average sd Region (lab)
Fat A	0,096	0,059	0,104	0,057
Fat B	0,097	0,053	0,071	0,053



	Mean d range Season (trial)	Average sd Season (trial)	Mean d range Region (lab)	Average sd Region (lab)
Crude Protein	0,111	0,053	0,077	0,046
True Protein	0,056	0,037	0,048	0,032

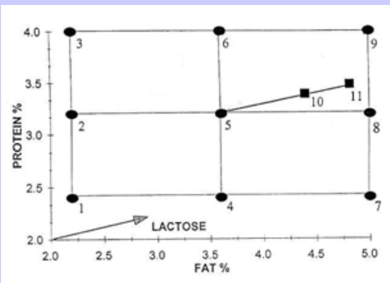
BCR MIR Prog. 1991:

15 European countries (labs)

8 trials on 1 year

2 bulk milks/trial/lab

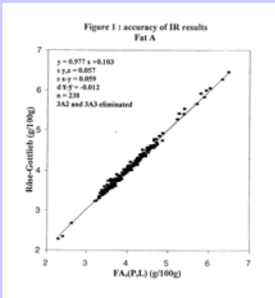
Appropriateness of recombined milk samples for centralised calibration

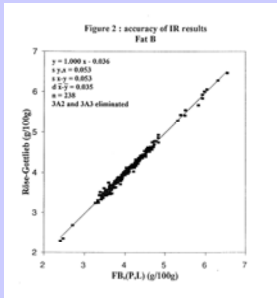


O. Leray, 1988, 1990, 1998, IDF 141

Example : BCR MIR European Programme 1991

15 labs (countries) x 2 bulk milks x 8 periods of 1 year

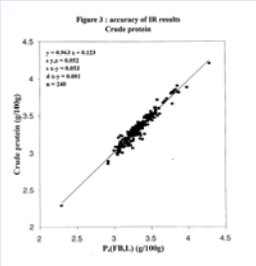


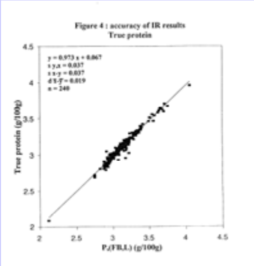


Calibration samples according to IDF 141

Example : BCR MIR European Programme 1991

15 labs (countries) x 2 bulk milks x 8 periods of 1 year

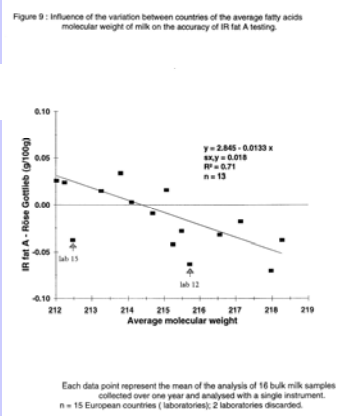




Calibration samples according to IDF 141

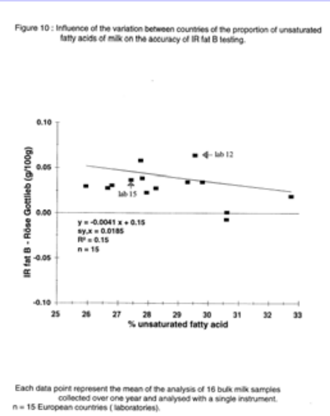
Matrix effects:

MW / Fat A



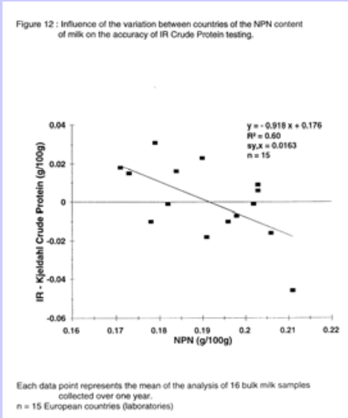
Matrix effects:

UFA / Fat B



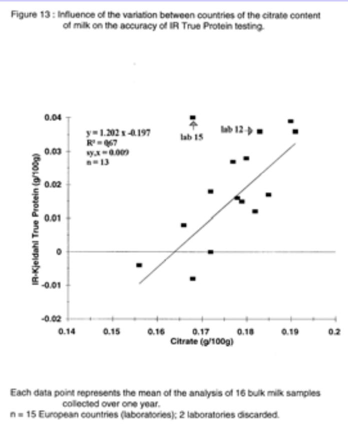
Matrix effects:

NPN / CP



Matrix effects:

Citrate / TP



Elements of choice for a centralised calibration system

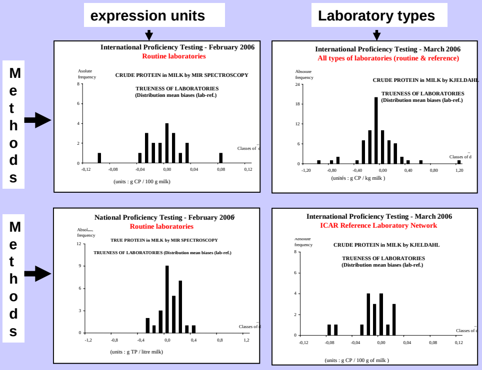
Comparison of of laboratory bias distributions in Proficiency Testing

- **In centralised calibration :**
  laboratory bias (1) = bias with the reference method (2) + calibration bias (3)
- **MIR PTs :** **measures the distribution of laboratory biases (1)=(2)+(3)**
- **PTs on reference methods :** **measures the distribution of biases with the reference method (2)**
- **Comparing the standard deviations (or ranges) of biases between labs :**
  Routine method SD ≤ reference method SD ⇒ Improvement or equivalence (4)
  Routine method SD > reference method SD ⇒ discrepancy of uncertainty (5)

(4) => Centralised calibration system applicable.

(5) => It is to users (milk recording) to consider whether or not the extent of discrepancy is acceptable for the intended use.

Example : Comparison of laboratory bias distributions



Conclusion on appropriateness

- Where applicable, centralised systems for reference and calibration are :
  - more convenient tools for laboratories
  - more securing systems for users
- Centralised calibration requires :
  - either routine methods insensitive to matrix effects
  - or to concern areas with negligible variation in matrix composition.
- Otherwise its applicability relates to :
  - the loss of precision accepted compared to the advantages of a centralisation of the reference.
  - the uncertainty of analytical results needed by users.

Conclusion

- Tools for the application of centralised calibration systems already exit and are published in ICAR Sessions proceedings:
  - Appropriate method for calibration sample preparation (RMs)
  - Means for mid (chemicals) and long term (deep freezing) preservation
  - Structure for reference values checking or determination (ICAR Ref Lab network)
- Centralised calibration can be also an answer to the question of checking/fitting calibration of in farm analytical devices...

Thank You for your attention !