Interlaboratory reference system & centralised calibration
Pre-requisites & standard optimum procedures

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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.
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 : \(\Rightarrow\) No / limited matrix effects \(\Rightarrow\) Overall accuracy with vs without centralised calibration ; matrix effects \(\Rightarrow\) choice

2 - Laboratory group : \(\Rightarrow\) homogeneity for methods, criterion expression, units

3 - Sample preservation : \(\Rightarrow\) Adequate to required shelf life

4 - Logistic : Sample transport facilities \(\Rightarrow\) safe, in time
### Mid infra red spectroscopy and matrix effects on classical wavelengths

<table>
<thead>
<tr>
<th>Components</th>
<th>Wavelength (µm)</th>
<th>Interferents corrected</th>
<th>Interferents uncorrected</th>
<th>Influencing factors</th>
<th>Origins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>4.2</td>
<td>Protein (Lactose)</td>
<td></td>
<td>FA Molecular weight</td>
<td>Fat, FA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ester linkage</td>
<td>Fat, FA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>breaking (lipolysis)</td>
<td>Fat, FA</td>
</tr>
<tr>
<td>Fat</td>
<td>2.8</td>
<td>Protein</td>
<td>Lactose</td>
<td>FT-MIR</td>
<td>Fat, FA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fat, FA</td>
</tr>
<tr>
<td>Protein</td>
<td>6.5</td>
<td>Fat</td>
<td>Lactose</td>
<td>FT-MIR</td>
<td>Protein, Lactose</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Protein, Lactose</td>
</tr>
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</table>

### BCR MIR Programme 1991 - Seasonal and regional effect - Comparison of Fat A and Fat B

<table>
<thead>
<tr>
<th>Value in g/100g</th>
<th>Season (trial)</th>
<th>Average ±sd (trial)</th>
<th>Region (lab)</th>
<th>Average ±sd (lab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat A</td>
<td>0.096</td>
<td>0.059</td>
<td>0.104</td>
<td>0.027</td>
</tr>
<tr>
<td>Fat B</td>
<td>0.097</td>
<td>0.053</td>
<td>0.071</td>
<td>0.020</td>
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</tbody>
</table>

### BCR MIR Programme 1991 - Seasonal and regional effect - Comparison of Crude Protein and True Protein

<table>
<thead>
<tr>
<th>Value in g/100g</th>
<th>Season (trial)</th>
<th>Average ±sd (trial)</th>
<th>Region (lab)</th>
<th>Average ±sd (lab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>0.111</td>
<td>0.053</td>
<td>0.077</td>
<td>0.046</td>
</tr>
<tr>
<td>True Protein</td>
<td>0.056</td>
<td>0.037</td>
<td>0.046</td>
<td>0.033</td>
</tr>
</tbody>
</table>
### Protein expression Crude vs True Protein

<table>
<thead>
<tr>
<th>Variation</th>
<th>Concentration</th>
<th>Range</th>
<th>Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasonal</td>
<td>0.14 - 0.22</td>
<td>0.08</td>
<td>INRA (BCR1992)</td>
</tr>
<tr>
<td></td>
<td>0.14 - 0.24</td>
<td>0.10</td>
<td>Cecalait (1992-1996)</td>
</tr>
<tr>
<td>Within region</td>
<td>-</td>
<td>0.05</td>
<td>Cecalait (1996)</td>
</tr>
<tr>
<td>Between regions (FR)</td>
<td>0.14 - 0.22</td>
<td>0.08</td>
<td>Cecalait (1996)</td>
</tr>
<tr>
<td>Between CE countries</td>
<td>0.17 - 0.21</td>
<td>0.04</td>
<td>INRA (BCR1992)</td>
</tr>
</tbody>
</table>

### 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 related towards national levels  
  ⇒ promote national PTs and CC
- 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)

1- Evaluation for choice of central calibration:
  a- Picture of current situation $\Rightarrow$ PTs (ref / routine)
  b- Evaluation of overall accuracy $\Rightarrow$ region & season effects

2 - Characteristics of calibration RMs:
  $\Rightarrow$ quality, safety, preservation, shelf life, fit-to-purpose

3 - Assign reference values $\Rightarrow$ laboratories, organisation

4 - Calibration $\Rightarrow$ pre-calibration, local correction
  $\Rightarrow$ external = PTs, internal = ISO 8196
CRUDE PROTEIN in MILK by KJELDAHL

TRUENESS OF LABORATORIES

(Distribution mean biases (lab-ref.)

Absolute frequency (unités : g CP / kg milk)

International Proficiency Testing - March 2006

All types of laboratories (routine & reference)

R e f e r e n c e

International Proficiency Testing - February 2006

Routine laboratories

Principle

(Quarterly) comparison thr. PTs:
- simultaneously
- same q samples (n repl.)
- same p laboratories
- local calibrations

For collective purpose:
1- sd_mkt = d_ref => OK
2- sd_mkt < sd_ref < √2 sd_ref
  => lab effect acceptable
3- √2 sd_mkt < sd_ref
  => discrepancy in overall accuracy

Decision : sd_mkt acceptable / not?

1a - Evaluation of current situation through PTs

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)

1b - Evaluation of overall accuracy in centralised calibration

Meeting of ICAR Reference Laboratory Network, 16 June 2008 - ICAR Session Niagara Falls 2008
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

<table>
<thead>
<tr>
<th>Region</th>
<th>Period</th>
<th>Period effect per region</th>
<th>Global analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d</td>
<td>s</td>
</tr>
<tr>
<td>i</td>
<td>1</td>
<td>d</td>
<td>s</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>d</td>
<td>s</td>
</tr>
<tr>
<td>...</td>
<td></td>
<td>d</td>
<td>s</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>d</td>
<td>s</td>
</tr>
</tbody>
</table>

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1 - **Physico-chemical quality of milk**:

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

- **Milk handling**: 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**

### 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

⇒ recombined milk samples in orthogonal experimental design
Example: Experimental design for MIR calibration (recombined samples)


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Example: Experimental design for SCC calibration (recombined samples)

Cecaille, CE Programme FAIR, 1997–1999

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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
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 $Y_L$ (ref) & $X_L$ (rout)

$$
Ref_c = Ref_R \cdot \left( \frac{Y_L}{X_L} \right) \quad \text{or} \quad Ref_c = Ref_R - \left( X_L - Y_L \right)
$$
Centring theoretical values for centralised calibration

Representative sample
Average of representative milk samples
Correction to reduce the mean bias of representative samples

Reference

Y = X

Analyser

Y

X

Y

X

$d = \bar{X} - \bar{Y}$

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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

$\Rightarrow$ Final correction:

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

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Local bias correction to reduce existing region effect

\[
Y = X - d_L = X - \frac{Y_l}{X_l}
\]

**Reference**

\[Y_l = \frac{Y_l}{X_l}\]

**Analyser**

\[X_l\]

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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
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!