Fourth ICAR
Reference Laboratory Network Meeting

Niagara Falls - USA
16 June 2008

MA SC

ICAR Sub-Committee on
Milk Analysis

FOREWORD

ICAR Reference Laboratory Network is now in existence for twelve years. It was established in order to constitute the basis for an international analytical quality assurance (AQA) system for milk recording. Many country members of ICAR took benefit of the network and the proficiency study schemes implemented for it to develop or improve their national AQA system, whereas others, which had none, may have the opportunity to implement one.

The first meeting of ICAR Reference Laboratory Network held in Interlaken in 2002 was the first opportunity for the members of the network to meet one another and have the possibility to establish links that could enable collaboration. In order to introduce the general scope of the network, an overview of analytical QA/QC systems in different ICAR member countries was given by several speakers.The valuable discussions and outcomes of the event triggered the interest to renew such a meeting at the occasion of every biennial ICAR Sessions. So was done in Sousse-Tunisia at the 34th ICAR Session in May-June 2004, where were dealt different issues on small ruminant milk analysis, method evaluation and ICAR interlaboratory proficiency studies, then, at the 35th ICAR Session in Kuopio-Finland in June 2006 where was introduced the ICAR certification policy, reference system and centralised calibration approaches and the discussion on accuracy needed for milk recording testing.

Year 2006 was identified as the end of the first period of the implementation/development of the AQA system of ICAR after ten years have passed from the launching of the laboratory network and twelve from the start-up of the implementation programme. From Kuopio, it as decided to produce practical guidances and tools in order to facilitate the work of reference and routine laboratories and harmonise practices in ICAR countries. This is the objective of the present meeting in Niagara Falls to present and detail what can be proposed for use to laboratories and how they can benefit of the network structuring model proposed by ICAR. Examples existing in different countries of three continents serve to illustrate and confirm the interest of pieces of theory and procedures prior presented in a first part.

We sincerely hope that the following contents can meet the interest of the members of the network and ICAR organisation members and help in further optimisation in analytical organisation and practices.

Poligny, 27th August 2008

Olivier Leray
Chair of ICAR Sub-Committee on Milk Analysis

CONTENTS

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Steve	Frank	United DHIA	United States
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First name	Last name	Organisation	Country
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Dave	Sukup	Heart of America DHIA	United States
Chris	Thompson	University of Kentucky	United States
Harrie	van den Bijgaart	Qlip NV	Netherlands
Simon	Vander Woude	High Desert Dairy Lab, Inc	United States
Deb	van de Water	CanWest DHI	Canada
Jan	Venneman	CRV Holding BV	Netherlands

Update on ICAR Reference Laboratory Network – Evolution since 1996

Olivier Leray

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History

A policy for analytical quality assurance (AQA) was introduced at the 29th ICAR Session in Ottawa in 1994 that should cover every aspect of milk recording analysis and can provide confidence to stakeholders, ensure equivalence of genetic evaluation and enable analytical system recognition between countries.

That policy was handled by the Working Group on Milk Testing Laboratories and from 2006 continued by the new Sub-Committee on Milk Analysis.

From 1994 the working group has defined essential guidelines so as to assure a minimum precision in milk recording analysis provided the recommendations are applied and, from 1996, created a network of expert laboratories expected to become the basis of an international analytical quality assurance system for milk recording, called ICAR Reference Laboratory Network.

The international reference laboratory network has become an essential piece of the AQA system aiming at analytical harmonisation as its members are entrusted to be intermediaries between national levels and the international level where optimum methods and practices are defined (IDF/ISO guides and standards, ICAR guidelines) to transmit adequate information to milk testing laboratories.

Structure and architecture

The international network constitutes a structure through which, thanks to interlaboratory studies, it becomes possible to provide an international anchorage to routine laboratories and estimating overall accuracy of milk recording measurement and absolute measurement uncertainty in individual laboratories.

This is realised through two levels of network implementation (possibly three), national (or regional) and international. The national reference laboratories operate as bridges for precision traceability between both national and international levels where interlaboratory studies are carried out respectively. A third layer can exist for instance in federal countries where as well regions can organise labs in network or could be developed in the future for on-farm analysis in the prospect of possible sub-network monitored by regional laboratories.

Membership

This makes that any laboratory commissioned to monitor routine testing laboratories should be invited by their national organisation to join the network. For specific situation where only few laboratories with no national co-ordination, individual routine laboratories may also join the network so as to benefit to a direct anchorage to the international level whereas, in well structured local situations, so-called reference laboratories can establish the junction between routine labs and the international level.

Competence and expertise requested as eligibility criteria to belong to the network are one or more of the followings :

1- National ring test organizer
2- Reference Material supplier
3- Master laboratory for centralized calibration
4- Teaching and training in laboratory techniques
5- Information on analytical methods
6- Evaluation of analytical methods/instruments
7- Research on analytical methods
8- National regulatory control of DHI analyses

the ideal situation being where the reference laboratory covers every competence item and therefore can ensure consistency and continuity in missions to routine laboratories.

Evolution

The numbers of laboratories qualified for various scientific/technical mission have increased gradually from 1996 to 2003, with its membership raised up to 38 members and since then it keeps stable about 38. In mid 2008 there are 38 of 32 countries involved in cow milk analysis, of which as well 16 work for goat milk and 14 for sheep milk.

Meanwhile the number of declared eligibility criteria continues to increase thus showing a qualitative development of the network towards maturity. In 2008 75% of competence items realised by 34% of members, and 50% by 63%.

Interlaboratory proficiency studies

Since 1996 an annual interlaboratory proficiency scheme has been regularly run twice a year for methods used as reference to calibrate routine methods for fat, protein and lactose in cow milk. It was complemented from 1999 with methods for methods for urea and somatic cell counting. In 2008 participant number is stable with about 20 for fat, 21 for protein, 1 for lactose, 15 for urea and 21 for SCC.

Significant improvement of analytical performances has been noted and today the overall precision observed within the network appears better than that of respective method standards thereby brings proofs of the efficiency of the scheme.

Stage of progress in AQA implementation with the network

The end of the first phase of implementation of the network was stated in ICAR Session in Kuopio 2006 and the launching of a second phase declared. As the general frame and architecture has been drawn and established time has come to feed the system with installing sustainable operations and activities for the benefit of harmonisation in ICAR member countries.

Proper models are to be given through guidelines to organise proficiency studies at national levels adequate for calibration purposes, define methodologies to orient and implement centralised calibration, evaluate analytical precision traceability, establish the international anchoring thanks to ICAR Reference Laboratory Network.

Beside education and training for laboratory practitioners should be promoted through the network with regard to analytical methods for milk and the respective former items and implemented at national levels based on international guidelines and standards.

Conclusion

The AQA system launched by ICAR in 1996 has already shown efficiency at the network member level. The analytical quality of national level remains under the responsibility of network members to which appropriate tools and guidance should be brought and developed where missing. The work has been undertaken by the Sub-Committee on Milk Analysis since 2006.

MA SC

ICAR Sub-Commitee on Milk Analysis

ICAR Reference Laboratory Network

- 4th Meeting, Niagara Falls, 16 June 2008

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- Agenda -

8.00 : Opening - Welcome - Round table for presentation

8.20 : Introduction : ICAR Reference Laboratory Network history and objectives
ICAR analytical strategy - International anchorage & harmonisation *(O. Leray, Cecalait, FR)*

8.50 : Interlaboratory reference systems and centralised calibration – Prerequisites and standard optimum procedures *(O. Leray, Cecalait, FR)*

9.10 : Discussion

9.40 : The way to reference systems and centralised calibration for milk recording testing - Present status in Germany *(C. Baumgartner, MPR, DE)*

10.00 : Health break

10.20 : Reference system and centralised calibration for milk recording testing in Argentina *(R. Castañeda, Inti-Lacteos, AR)*

10.40: Reference system and centralised calibration for milk (payment) testing in USA, *(D. Barbano, Cornell University, USA)*

11.00 : Assessment of laboratory performances and analytical equivalence in milk testing in North America, *(P. Sauvé, Canadian Laboratory Services, CA)*

11.30 : Discussion

12.00 - Closure of the meeting

- INTRODUCTION - GENERAL OBJECTIVES -

History : **ICAR Session in Ottawa1994,**
=> Analytical Quality Assurance (AQA) policy by ICAR

General objective : Develop an **international AQA system for DHI** based on harmonised laboratory practices.

Goal : **Confidence, equivalence, comparability**
=> within / between countries,
=> worldwide : international genetic evaluation.

Implementation by MA SC (MTL WG) :
> Guidelines for the harmonisation of analytical practices :
Analytical methods, Quality Assurance,

> International network of reference laboratories for milk recording analytical performances

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ROLES OF THE LABORATORY NETWORK

ICAR Reference Laboratory Network is expected to operate as **an** international platform for milk recording as to

- diffuse/promote GLP and AQA based on international guides and standards => communication *(Internet, website)*

- provide precision traceability and anchorage to consensual international "true values" to routine labs via network members
=> analytical data harmonisation *(PTs, RMs)*

- a mean for developing collaborations for laboratory purposes
=> Co-operation *(Education, training)*

Model & explanation provided every year to ICAR member organisations

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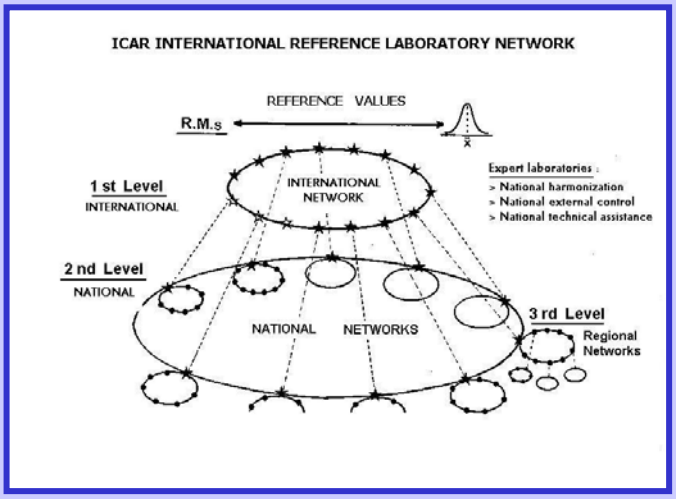
THEORETICAL STRUCTURE



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Missions / activities expected - Eligibility criteria -

- 1- National ring test organizer
- 2- Reference Material supplier
- 3- Master laboratory for centralized calibration
- 4- Teaching and training in laboratory techniques
- 5- Information on analytical methods
- 6- Evaluation of analytical methods/instruments
- 7- Research on analytical methods
- 8- National regulatory control of analyses
- 9- Routine testing where only 1 or 2 labs/country

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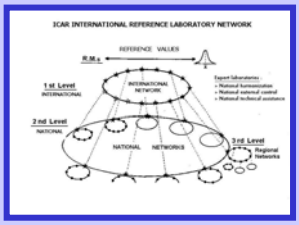
ICAR Reference Laboratory Network

Composition & evolution

from 1998 to 2008



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ICAR Reference Laboratory Network

Membership in 2008

38 laboratory members from **32** countries as follows:

Argentina	(1)	Austria	(1)	Belgium	(2)	Canada	(1)
Cyprus	(1)	Czech Republic	(1)	Denmark	(1)	Estonia	(1)
Finland	(1)	France	(1)	Germany	(1)	Hungary	(1)
Ireland	(1)	Israel	(1)	Italy	(1)	Korea	(1)
Latvia	(2)	Lithuania	(1)	The Netherlands	(1)	New Zealand	(1)
Norway	(1)	Poland	(1)	Slovak Repub.	(1)	Slovenia	(1)
South Africa	(3)	Spain	(1)	Sweden	(1)	Switzerland	(1)
Tunisia	(2)	United Kingdom	(1)	U.S.A.	(2)	Zimbabwe	(1)

(n) : number of member(s)

among which : 38 members for cow
16 members for goat
14 members for sheep

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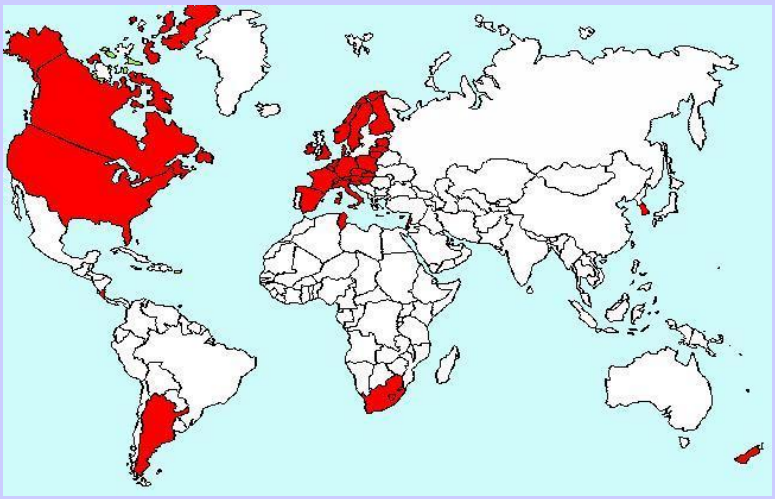

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ICAR Reference Laboratory Network - Evolution since 1998 -

Evolution of the composition and national roles from 1998 to 2007 (end of year)

YEAR	NRTO	RMS	MLCC	TLT	IAM	EAMI	RAM	NRCA	DHIA	PAYMENT	Other anal.	Members
1998	15	16	13	13	16	1	11	2	2	1	1	23
1999	17	18	17	14	17	1	12	2	3	1	1	28
2000	16	21	19	15	19	1	13	3	5	1	1	33
2001	19	22	19	18	21	3	15	5	6	2	1	35
2002	20	23	19	19	23	8	15	8	11	5	1	37
2003	21	26	19	21	24	12	16	9	14	7	3	38
2003	21	26	19	21	24	12	16	9	14	7	3	38
2004	25	26	18	20	24	14	16	9	16	9	3	38
2005	24	24	17	19	22	13	15	10	15	8	3	37
2006	24	24	17	20	22	14	15	10	15	10	3	36
2007	22	24	18	22	24	17	17	13	17	13	3	38

NRTO = National Ring Test Organiser
TLT = Training in Laboratory Techniques
RAM = Research on Analytical Methods
Membership = Officially nominated by ICAR National Committees

RMS = Reference Material Supplier
IAM = Information on Analytical Methods
NRCA = National Regulatory Control of Analyses

MLCC = Master Laboratory for Centralised Calibratio
EAMI = Evaluation of Analytical Methods/Instruments
DHIA = Dairy Herd Improvement Analyses
Payment = Analyses for milk payment

Evolution of the proportions of national roles from 1998 to 2007 (end of year)

YEAR	NRTO	RMS	MLCC	TLT	IAM	EAMI	RAM	NRCA	DHIA	PAYMENT	Other anal.	Members
1998	68	73	59	59	73	5	50	9	9	5	5	100
1999	63	67	63	52	63	4	44	7	11	4	4	100
2000	48	64	58	45	58	3	39	9	15	3	3	100
2001	54	63	54	51	60	9	43	14	17	6	3	100
2002	54	62	51	51	62	22	41	22	30	14	3	100
2003	55	68	50	55	63	32	42	24	37	18	8	100
2004	66	68	47	53	63	37	42	24	42	24	8	100
2005	65	65	46	51	59	35	41	27	41	22	8	100
2006	67	67	47	56	61	39	42	28	42	28	8	100
2007	58	63	47	58	63	45	45	34	45	34	8	100

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Eligibility criteria declared in 2008

Criteria number N	Proportion %	Lab number with N	Lab % with N	Lab number with at least N	Lab % with at least N
8	100%	5	13%	5	13%
7	88%	4	11%	9	24%
6	75%	4	11%	13	34%
5	63%	4	11%	17	45%
4	50%	7	18%	24	63%
3	38%	3	8%	27	71%
2	25%	2	5%	29	76%
1	13%	4	11%	33	87%
0	0%	5	13%	38	100%

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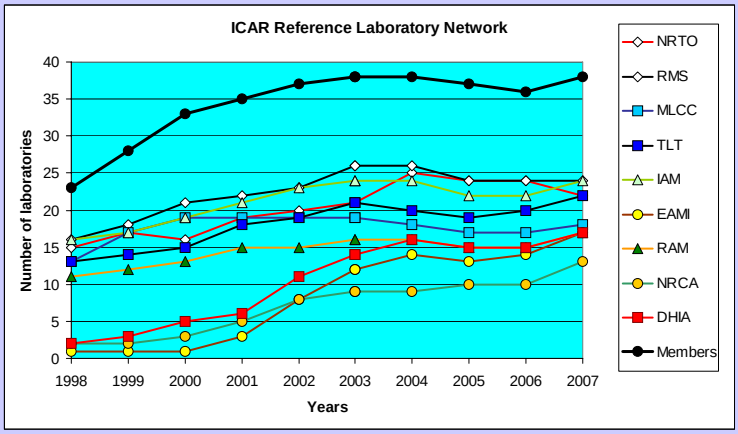
Evolution of membership and missions/activities from 1998 to 2008



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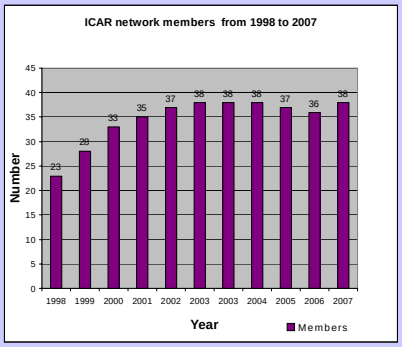

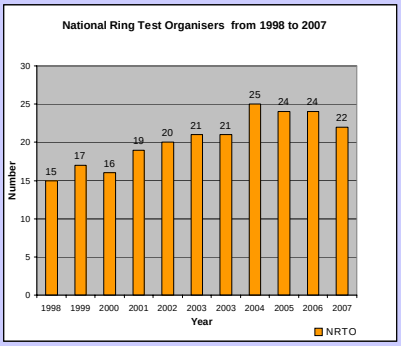

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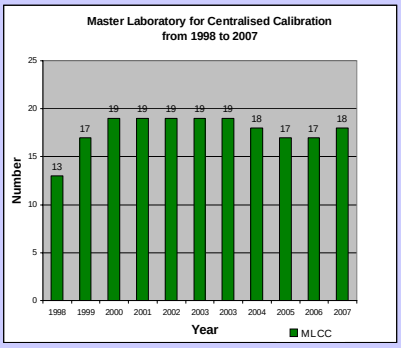
Evolution of membership and missions/activities from 1998 to 2008



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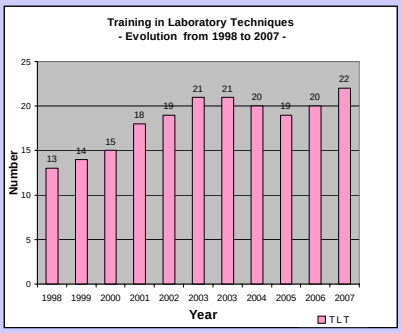

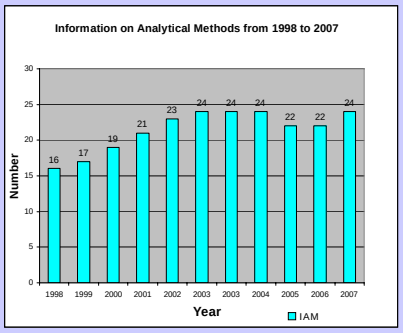

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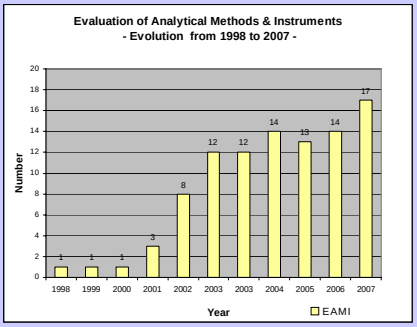

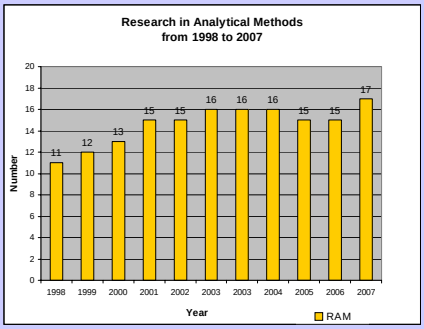

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International interlaboratory proficiency studies

From 1996 :	International proficiency scheme organised by ICAR
Frequency :	twice a year
Participants :	members of ICAR ref lab Network
Analytical methods :	- reference methods to calibrate routine methods for fat, protein and lactose
	- methods for urea somatic cell counting
Type of milk :	cow milk

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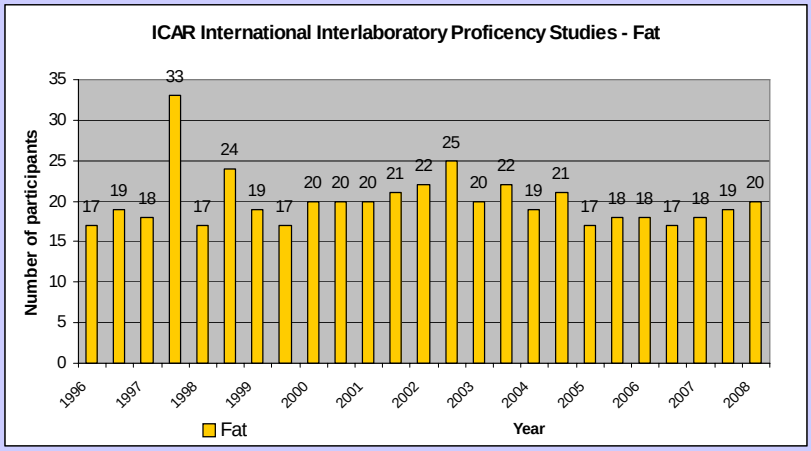
Participation in international proficiency studies from 1998 to 2008



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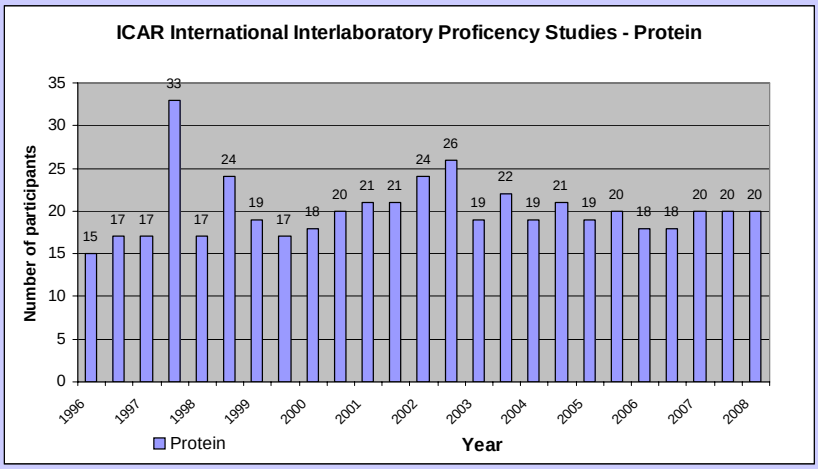

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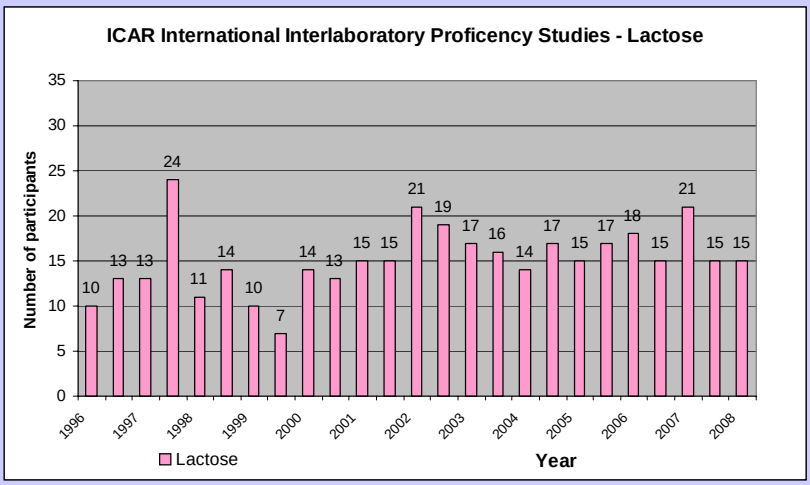

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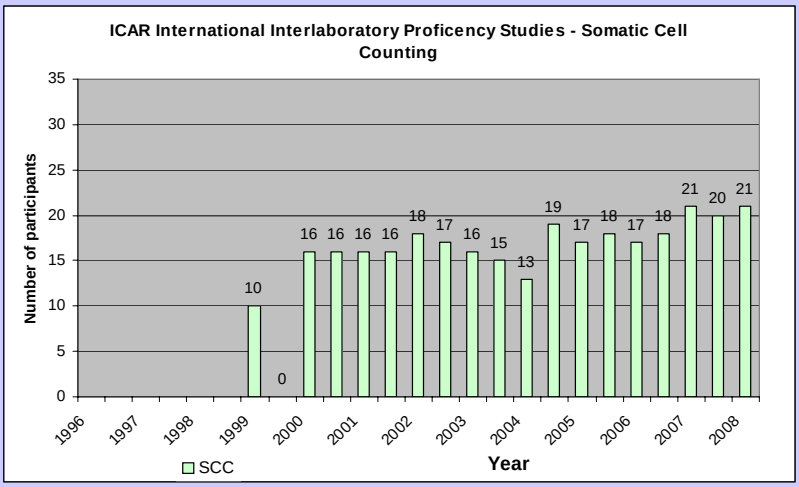

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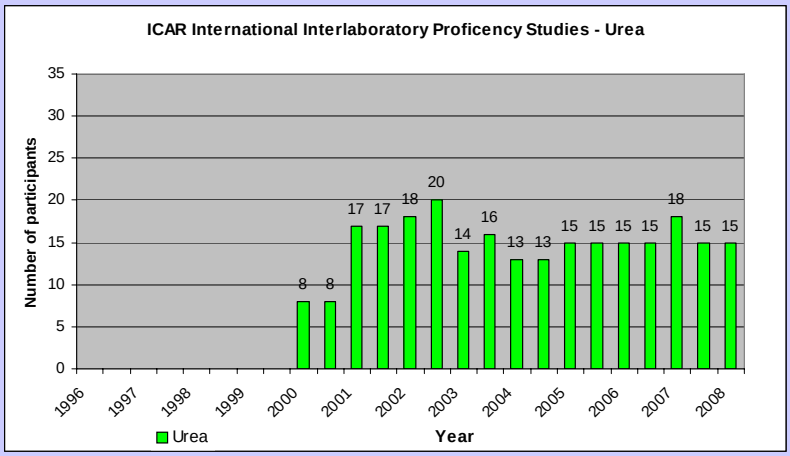

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CONCLUSION ON THE NETWORK IMPLEMENTATION

Nominations by national organisations :

- **Number :** Stability from 2003 around 38 members
  ⇒ growth completed

- **Qualification :** Increase of mission numbers (eligibility criteria)

International Proficiency Testing schemes :

- Regular participation of about 50% of laboratory network members

- Improvement of performance from 2003

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ICAR AQA strategy – International anchorage and harmonisation

Olivier Leray

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Introduction

The policy for analytical quality assurance (AQA) implemented from 1994 has been based on the harmonisation of laboratory practices and analytical performance laboratories in ICAR member countries thanks to missions devoted to expert laboratories, so-called reference laboratories as justified by strong technical competence. Those missions refer to lab monitoring, expertise and service supply for quality assurance (QA) and quality control (QC). ICAR countries have been invited to nominate or create minimum one such a laboratory for national milk recording so that the whole of reference laboratories can become members of an active international reference laboratory network.

The international reference laboratory network has become an essential piece of the AQA system aiming at analytical harmonisation as its members are entrusted to be intermediaries between national levels and the international level where optimum methods and practices are defined (IDF/ISO guides and standards, ICAR guidelines) to transmit adequate information to milk testing laboratories.

International anchorage

International anchorage is made :

- First, through same/similar practices in every ICAR countries which is achieved through using same international standards and guides,
- second, establishing concrete technical links between the high level of expertise (international reference laboratories) and routine testing laboratories in every country.

A technical linkage is to be made between the unknown truth given by the consensus of international milk recording community – represented by average results produced by the international reference laboratory network – and the final results obtained by testing laboratories.

It can be achieved through two major tools that can be implemented in parallel at both national and international levels with proper connection, correspondence and relay.

- Interlaboratory proficiency schemes : measuring lab performances. Through adequate combination, it is possible to establish and measure the chaining of errors in analytical steps that contribute to the final result (with regard to the reference methods and routine methods).

- Interlaboratory certification schemes : determination of true (or reference) values for reference materials (RMs).

Performance evaluation

A protocol was adopted by ICAR for regular ICAR proficiency studies – international level – and is to be proposed in guidelines to ICAR countries for national implementation. Trials use q=10 samples evenly distributed throughout the concentration range of usual milk analyser calibration and labs perform duplicate analyses (n=2). Lab evaluation is made through the laboratory bias - average of differences $\bar{d}_{Lk}$ measured between lab results and the reference value $\bar{X}_S$ (grand mean of all the labs per sample) - and the standard deviation of differences as an indicator of consistence (outlier result).
Laboratory score $\bar{d}_L = \sum \bar{d}_{Lk} / q = \bar{x}_L - \bar{X}$ must lay within the limits associated to uncertainty of $\bar{d}_L$

$U_{\bar{d}L} = \pm 2.(U_{\bar{x}L}^{\ 2} + U_{\bar{X}}^{\ 2})^{1/2}$ or $U_{\bar{d}L} = \pm 2.[(\sigma_R^{\ 2}-\sigma_r^{\ 2}.(1-1/nq).(1+1/p)]^{1/2}$

Beside punctual elements of within lab reproducibility can be estimated through

$sR_L^2 = sr^2 .(1-1/n) + \bar{d}_L^2 + sd^2$

with estimate calculated by averaging precision elements of several successive trials. Within lab reproducibility standard deviation can then be used for determining uncertainty of test results.

Result uncertainty

Precision and accuracy elements produced through PT results and analyser monitoring and calibration allow to calculate the overall accuracy and uncertainty of routine testing results in a laboratory. To realise adequate estimation elements used are to be obtained from sufficient numerous data.

For the reference methods (q samples and n replicates) it is calculated according to ISO 5725-6 as

$U_{ref} = \pm u_{0.975} .[sR_{L,ref}^2 - sr_{L,ref}^2.(1-1/nq)]^{1/2}$ and with high nq (calibration) $U_{ref} \approx \pm u_{0.975} .(sR_{L,ref}^2 - sr_{L,ref}^2)^{1/2}$

For routine (alternative) methods, it is estimated according to ISO 8196 through
$U_{alt} \approx \pm u_{0.975} . (sR_{L,alt}^2 + s_{y,x}^2)^{1/2}$

Overall uncertainty of routine testing results U is obtained by combining both types of error as
$U \approx \pm u_{0.975} .(sR_{L,ref}^2 - sr_{L,ref}^2 + sR_{L,alt}^2 + s_{y,x}^2)^{1/2}$

Traceability to an international reference

The reference laboratory of every national laboratory network participates in national and international proficiency studies in parallel. Special training and procedures to ensure trueness and performance stability what is checked through international PT results (re qualification of reference laboratories).

The bridge between national and international levels is calculated through the difference Δ between national and international references of parallel trials (Figure 2). The latter difference is calculated through the scores obtained by the reference laboratory M (master) in one and the other trials $\Delta = \bar{d}_{MN} - \bar{d}_{MI}$ provided laboratory bias is shown constant (established by several successive international PTs).
Since then any laboratory L can estimate a **virtual equivalent international score** from its national score by subtracting Δ : $\bar{d}_{LI} = \bar{d}_{LN} - \Delta$

Uncertainty of the estimate must take into account several steps involved in bridging so it is larger than a direct performance evaluation.

$U_{\bar{d}\,LI} = \pm 2.[(\sigma_R^2 - \sigma_r^2.(1-1/nq).(3+1/p)]^{1/2}$

- with large nq and p (labs) values : $U_{\bar{d}LI} \approx \pm 2.\sqrt{3}.(\sigma_R^2 - \sigma_r^2)^{½}$
- with highly qualified master laboratories ($\sigma_{RM} \approx \sigma_{rM}$) and large nq and p values : $U_{\bar{d}LI} \approx \pm 2.(\sigma_R^2 - \sigma_r^2)^{½}$

Comparison between laboratories

At a single level national or international it is easily realised through the difference $\bar{d}_{1,2}$ of scores of respective laboratories L1 and L2 (Figure 1), since $\bar{d}_{1,2} = \bar{x}_{L1} - \bar{x}_{L2} = \bar{d}_{L1} - \bar{d}_{L2}$

It is expected to stay between $\pm 2.\sqrt{2}\, U_{\bar{d}L} = \pm 2.[(\sigma_R^2 - \sigma_r^2.(1-1/nq).(2+1/p_1+1/p_2)]^{1/2} \approx \pm 2.\sqrt{2}.(\sigma_R^2 - \sigma_r^2)^{1/2}$

Between different trials, a **virtual equivalent international differences** can be estimated provided reference laboratories can establish correspondence to the international level (Figure 3) where the virtual difference can be calculated by

$D = \bar{d}_{LI1} - \bar{d}_{LI2} = (\bar{d}_{LN1} - \bar{d}_{LN2}) - (\Delta_1 - \Delta_2)$

With $\Delta_1 = \bar{d}_{MN1} - \bar{d}_{MI1}$ the bias of the reference of Trial 1 to that of the international trial
$\Delta_2 = \bar{d}_{MN2} - \bar{d}_{MI2}$ the bias of the reference of Trial 2 to that of the international trial

Uncertainty of the difference must take into account the several steps involved in bridging for two national networks and is calculated from uncertainty of the uncertainty of international correspondence formerly mentioned through $\pm 2.\sqrt{2}.\ U_{\bar{d}\,LI} = U_{\bar{d}LI} \approx \pm 2.\sqrt{6}.(\sigma_R^2 - \sigma_r^2)^{½}$ and with highly qualified master laboratories ($\sigma_{RM} \approx \sigma_{rM}$) $U_{\bar{d}LI} \approx \pm 2.\sqrt{2}.(\sigma_R^2 - \sigma_r^2)^{1/2}$

Certification of reference materials

Same type of trials as PT studies can be used to determine true value for reference materials provided the experimental design permit so.

ICAR protocol fit for purpose since proficiency testing and possible reference material are made to assess reference method and/or calibrate routine methods. For that reason it is recommended the sample number must be the same as that used for calibration. The minimum stated in ISO 8196 is 9. Guidelines are to be develop with this respect in the future.

Thanks to performance evaluation through proficiency studies, it is possible to select best performing laboratories to establish true (reference) values for RMs. Otherwise the whole of participants can be used provided proper discarding of outlier results and laboratories.

ISO 5725 provides adequate recommendation for calculation of true values and the associated uncertainty valid for both lab performance evaluation studies and reference material certification studies.

Conclusion

International laboratory anchorage passes through interlaboratory studies organised for dedicated laboratory network implemented on national and international level. Connection between levels is established by expert laboratories members of networks at both levels.

Technical tools already exist to take full benefit of the system developed other are to be developed from the theory and prospects above presented.

ICAR Reference Laboratory Network is the corner of the system and must be enhance with increased worldwide representativeness and competence of members.

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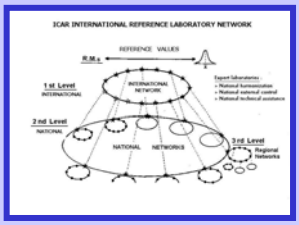
ICAR AQA Strategy

International anchorage & harmonisation

Olivier Leray, Cecalait, France



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ICAR analytical anchorage

Intent

> to establish links from local/national/regional levels to the international level

> to harmonise laboratories on a international collective reference

Means

> Guidelines, standards, GLP, AQA

> Interlaboratory proficiency studies ⇒ lab trueness traceability

> Reference materials ⇒ trueness improvement

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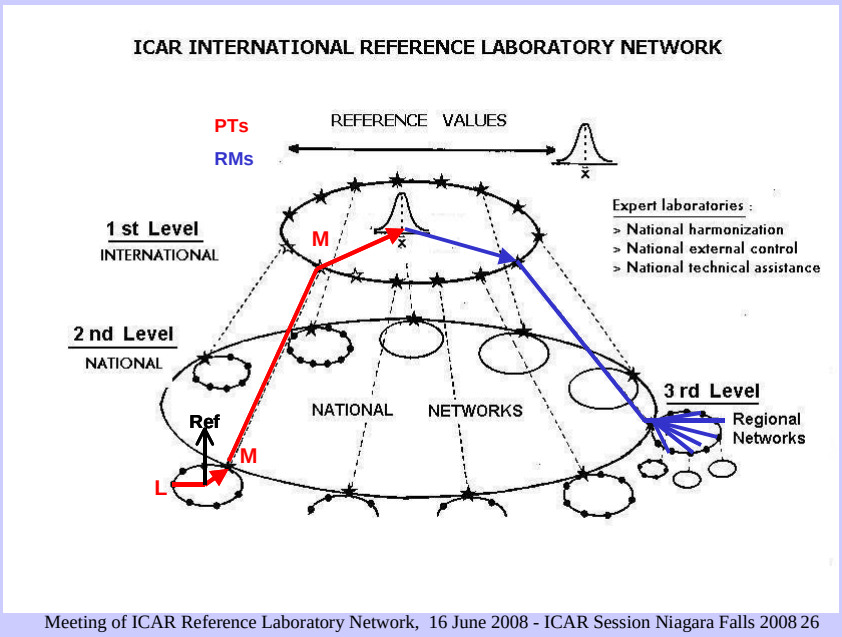

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Requirements for the reference

1- Technical :

⇒ Use of international reference methods (IDF/ISO)

⇒ Compliance with precision figures of the methods

2- Statistical : Unbiased and low uncertainty

⇒ sufficient number, representativeness of participants

3- Political/economical : recognition for the purpose

⇒ consensus of participants / organisations based on representativeness

For international genetic evaluation (Interbull), it should be built from results of laboratories from different countries !!!

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Possible uses of interlaboratory proficiency studies

1- Measuring laboratory performance

2- Measuring result uncertainty

3- Comparing laboratories (assess equivalence)

4- Providing traceability to international reference

5- Qualifying/selecting reference/expert laboratories

6- Assessing/certifying reference materials

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1- Measuring laboratory performance

Laboratory L

- participates with p laboratories, q samples in n replicates
- the estimate of sample S true value is $\bar{X}_S$
- means of n replicate (average) are $\bar{x}_{Lk}$
- level score (individual bias) is $d_{Lk} = \bar{x}_{Lk} - \bar{X}_S$

Laboratory score = Average of q level scores :

$$\bar{d}_L = \Sigma\, \bar{d}_{Lk} / q \quad \text{also} \quad \bar{d}_L = \bar{x}_L - \bar{X}$$

Additionally:

- standard deviation of repeatability sr_L
- standard deviation of differences sd_L
- Euclidian distance (equivalent to SEP) $D = (\bar{d}_L^{\,2} + sd^{\,2})^{1/2}$

Within lab reproducibility : $sR_L^{\,2} = sr^{\,2}.(1-1/n) + \bar{d}_L^{\,2} + sd^{\,2}$

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Evaluation example : ICAR PT scheme (10 samples in duplicates)

DETERMINATION of FAT in RAW (cow) MILK - page 1/6

Tableau I : Ranking of the laboratories

Nb	%	N°	d	Sd	D
1	5	1	+ 0,002	0,004	0,004
2	10	6	+ 0,001	0,005	0,005
3	15	8	+ 0,003	0,004	0,005
4	20	18	+ 0,004	0,004	0,005
5	25	3	- 0,002	0,005	0,005
6	30	13	- 0,000	0,005	0,005
7	35	10	- 0,001	0,005	0,005
8	40	12	- 0,006	0,005	0,008
9	45	2	+ 0,004	0,008	0,009
10	50	20	- 0,007	0,007	0,010
11	55	14	+ 0,007	0,007	0,010
12	60	17	+ 0,009	0,005	0,010
13	65	15	+ 0,005	0,010	0,011
14	70	11	- 0,009	0,007	0,012
15	75	7	+ 0,009	0,009	0,013
16	80	16	+ 0,002	0,013	0,013
17	85	5	+ 0,011	0,007	0,014
18	90	9	+ 0,001	0,017	0,017
19	95	19	- 0,023	0,008	0,024
20	100	4	- 0,023	0,012	0,026

(NC : OUT of RANKING because of insufficient data number)
(Nb : laboratory rank; % : relative rank)
(N° : laboratory identification number)
(d et Sd : mean and standard deviation of the differences (laboratory -reference))
(D : Euclidian distance to YX-axis origin = SQUARE ROOT.(d^2 + Sd^2))

Units : g / 100 g

The table should be studied in parallel with figure 2 where the laboratories are located according to an acceptability area (or target) the limits of which are :

$\pm$ 0,02 g / 100 g for $\bar{d}$ and 0,03 g / 100 g for Sd

The reference values are the average values of 20 laboratories having used the extraction method and after outlier discarding using Grubbs test at 5 % risk level.

Note : Limits are only indicative and so far do not constitute standard values; they indicate what is normally reachable by labs for their self evaluation.

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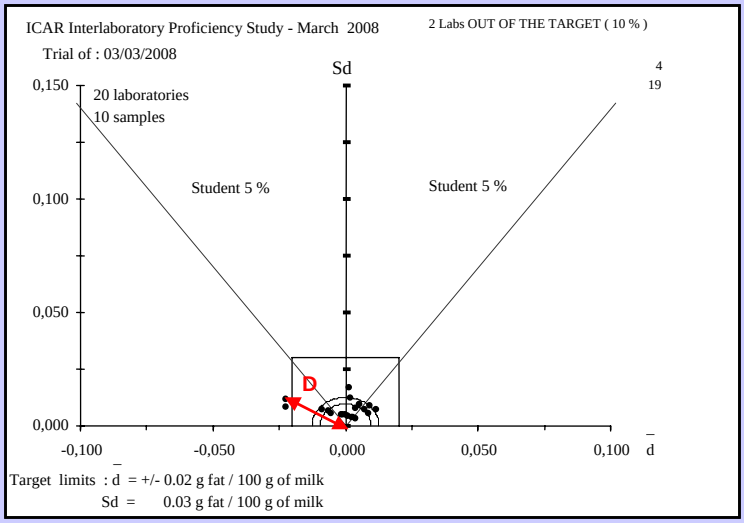
Evaluation example : ICAR PT scheme (10 samples in duplicates)



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2- Measuring test result uncertainty

Estimation from several ($N_t \geq 8$) last successive PT trials

ISO 5725-2: *(replacing laboratory variable by trials)*
Precision of Laboratory L ⇒ $\mathbf{sr_{L,ref}}$ and $\mathbf{sR_{L,ref}}$

ISO 5725-6: Reference method for *q samples and n replicates*

uncertainty $= \pm u_{0.975} .[sR_{L,ref}^2 - sr_{L,ref}^2.(1-1/nq)]^{1/2}$

(in calibration) $\approx \pm u_{0.975} .(sR_{L,ref}^2 - sr_{L,ref}^2)^{1/2}$ (1)

ISO 8196: Routine (alternative) method

uncertainty $\approx \pm u_{0.975} . (sR_{L,alt}^2 + s_{y,x}^2)^{1/2}$ (2)

From (1) + (2) ⇒ Overall uncertainty of routine testing results

$$\approx \pm u_{0.975} .(sR_{L,ref}^2 - sr_{L,ref}^2 + sR_{L,alt}^2 + s_{y,x}^2)^{1/2}$$

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3- Comparing laboratories

Same PT study

With scores of laboratories L1 and L2

$$\bar{d}_{L1} = \bar{x}_{L1} - \bar{X} \quad \text{and} \quad \bar{d}_{L2} = \bar{x}_{L2} - \bar{X}$$

Between lab performance comparison is made through the difference

$$\bar{d}_{1,2} = \bar{x}_{L1} - \bar{x}_{L2} \quad \Leftrightarrow \quad \bar{d}_{1,2} = \bar{d}_{L1} - \bar{d}_{L2}$$

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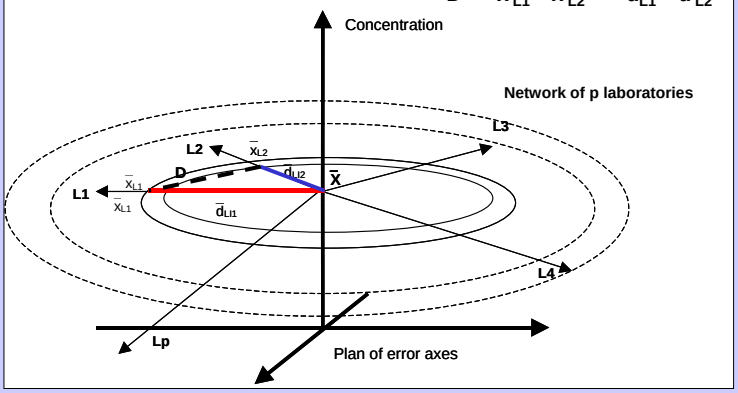
Comparison between laboratories L_1 & L_2

Figure 1 – Between laboratory comparison through an interlaboratory study

$$D = \bar{x}_{L1} - \bar{x}_{L2} = \bar{d}_{L1} - \bar{d}_{L2}$$



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4- International laboratory anchorage

Parallel national and international PT studies

Thanks to scores of the reference laboratory M

in national study $\bar{d}_{MN}$

in international study $\bar{d}_{MI}$

the virtual error between reference $\Delta = \bar{d}_{MN} - \bar{d}_{MI}$

the effective score of Lab L in the national study $\bar{d}_{LN} = \bar{x}_L - \bar{X}_N$

the **virtual international score of Laboratory L** is

$$\bar{d}_{LI} = \bar{d}_{LN} - \Delta = \bar{d}_{LN} - \bar{d}_{MN} + \bar{d}_{MI}$$

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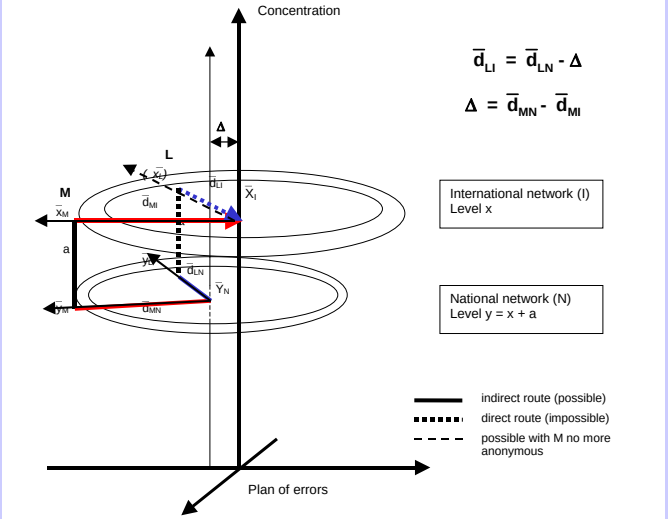
4- International laboratory anchorage



3- Indirect laboratory comparison

Different PT studies

Thanks to scores of reference laboratories M1 and M2

in national studies $\bar{d}_{MN1}$ and $\bar{d}_{MN2}$

in international studies $\bar{d}_{MI1}$ and $\bar{d}_{MI2}$

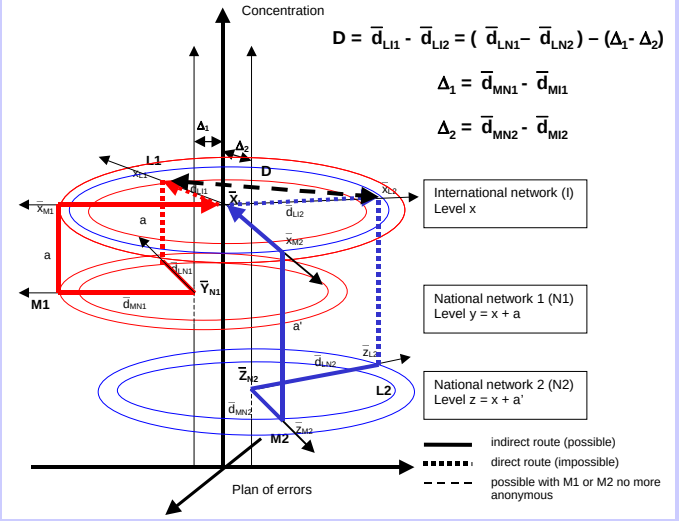
the virtual bias between reference $\Delta_1 = \bar{d}_{MN1} - \bar{d}_{MI1}$ and $\Delta_2 = \bar{d}_{MN2} - \bar{d}_{MI2}$

the effective scores in national studies $\bar{d}_{LN1} = \bar{x}_{L1} - \bar{X}_{N1}$ and $\bar{d}_{L2} = \bar{x}_{L2} - \bar{X}_{N2}$

the **virtual international difference between laboratories L1 and L2** is

$$D = \bar{d}_{LI1} - \bar{d}_{LI2} = (\bar{d}_{LN1} - \bar{d}_{LN2}) - (\Delta_1 - \Delta_2)$$

Comparison between laboratories L_1 & L_2



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5- Qualifying/selecting reference laboratories

Required regular good performance in PTs

RMs certification :

> Regular score compliance in a number of successive trials

Laboratory anchorage :

> Regular score compliance throughout time

> Constant bias (better 0) ⇔ $sR_{L,ref} \approx sr_{L,ref}$

Means of success : Trueness adequacy and stability ensured through RMs and special training, competence, caution.

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6- Assessing/certifying reference materials

Focus is given to reference values determination and reference material quality

ICAR protocol :

> Experimental design for PTs also possible for RMs

> Both tools are dedicated to calibration :
⇒ same concentration ranges
⇒ same sample numbers

Combined use is possible provided respective specific caution :

- Reference values: according to **ISO 5725-4** with uncertainty

- Laboratories : Qualified / selected on performance for the lowest uncertainty

- Experimental design: Consider long term homogeneity/stability

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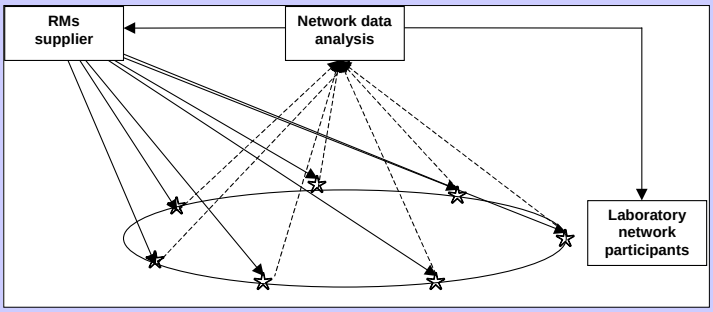
Example : Central RM system



General model : Numerous laboratories and samples ; robust reference

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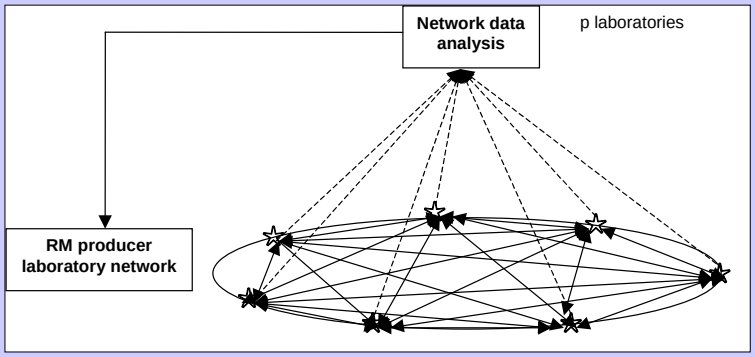
Example : Multiple RM system = crossed system



Specific model : Homogeneous laboratory groups ; numbers of laboratories and samples limited ; good performance must compensate small laboratory number ; consensus on group reference better than individual

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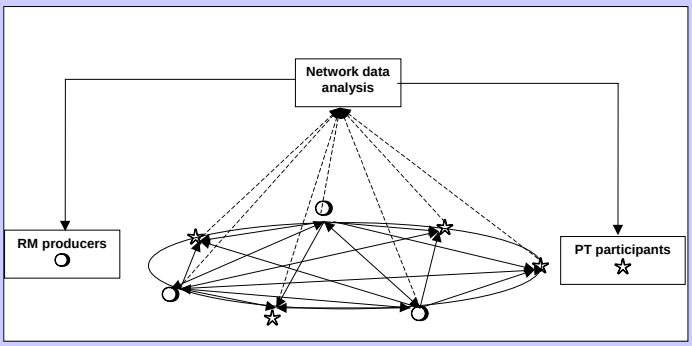
Example: Mixed system



Intermediate model : Heterogeneous laboratory groups ; a few laboratories address samples to a larger group ; samples number still limited ; more robust reference

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Conclusion

International anchorage

can provide objective elements on :

- the overall accuracy & uncertainty of milk testing
- the (degree of) analytical equivalence within ICAR

⇓

ICAR International Reference Laboratory Network

corner stone for analytical harmonisation
in milk recording

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Interlaboratory reference system and centralised calibration - Prerequisites and standard optimum procedures

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Introduction

Genetic evaluation on milk composition has become possible only through the generalised use of rapid automated method of milk analysis. Mid infra red spectroscopic methods and fluoro-opto-electronic methods have become predominant till being the only techniques used in large milk routine testing for milk composition and somatic cell counting.

For such methods calibration of routine methods is the key operation but also the most expensive to laboratories as it requires a lot of time and competence in sample preparation and reference analysis.

Sharing calibration cost between several laboratories thus amortising over many milk sample testing appears an economical alternative for laboratories beside the possibility to optimise both calibration sample quality and harmonise reference results through same values to all the laboratories. Indeed such calibration system can be easily associated to interlaboratory studies in order to optimise of the trueness of reference values for calibration.

Promoting the implementation of robust reference established by laboratory groups and centralised calibration in so-called reference system has become a new objectives of ICAR from 2006. Recommendations as pre-requisites and optimum procedures for implementation are needed for international harmonisation.

Objectives and prerequisites

The objectives are to establish reference values for an appropriate material (milk) that can be valid for a community of laboratories (spread over a collect territory), transfer consensus reference values to the laboratories to calibrate routine methods and at end assess effectiveness of the system.

Prerequisites refer to the accuracy of routine methods, the harmonisation of laboratories, appropriate logistic conditions.
- Depending on the variation of milk composition in the collect areas and the sensitivity of methods to matrix effects, the accuracy value can become larger than in usual calibration. Before implementing centralised calibration it is of major importance to evaluate whether or not the extent of accuracy is acceptable for the intended purpose i.e. milk recording. With this respect it can be referred to experiment results presented in Kuopio (O. Leray 2006).
- Methods, expression units (e.g. m/m, m/v, per 100 or per Kilo), criterion expression (e.g. True protein vs Crude protein) should be harmonised within the laboratory group so that calibration sample characteristics suit to every instrument equally.
- Sample preservation and transportation facilities should be adequate to analyse sample within short delays with no change in the physicochemical composition of calibration milk samples.

Means and tools

To achieve its goal, ICAR intends to produce suitable guidelines for laboratories on organising interlaboratory proficiency studies (PT) and centralised calibration (CC) and provide relevant services to countries.
International PT services are already supplied for the sake of the international reference harmonisation through a reference laboratory network according to a protocol approved by ICAR. This protocol should be detailed and become part of ICAR guidelines.
Developing/certifying international reference materials as gold standards is part of ICAR strategy beside promoting the use of national/local reference materials to relay international standard in countries either for checking reference methods or calibration.

Guidelines for proficiency studies

They will be in agreement with other general international standard on the subject which would be referred to but will additionally include specific requirements related to calibration and alternative methods thus establishing consistency with ISO 8196.

Especially
- the experimental design will be well stated with minimum numbers (e.g. 9 samples, 3 levels, 2 replicates) and concentration arrangement for optimised assessment (according to ISO 9622),
- standard statistical analysis and presentation recommended using performance scores and target figures. Slope, linearity, interactions assessment will complete the statistical analysis for studies with routine methods. Examples were published in ICAR Session proceedings in Rotorua (1998) and the IDF Bulletin 342/1999.

Guidelines for centralised calibration

Guidelines will indicate protocols to
- evaluate the overall accuracy in a centralised calibration system,
- define the characteristics of calibration reference material,
- assign reference values,
- provide indications for line adjustment in the laboratory.

Evaluation of the overall accuracy of a centralised calibration system

It can be performed by two ways, either once prior implementation of the system through an experiment provided natural conditions would not later, or through regular proficiency studies involving reference and routine methods.
Two protocols can be proposed depending on the situation
a- In-lab experiment : It is carried out prior implementation with a unique instrument provided pre-required condition of harmonisation will be maintained later. A number of representative samples are collected in milk testing lab areas and analysed by the experimenting laboratory for both reference and routine methods. Operations are evenly repeated throughout a campaign of milk production and regional and seasonal effect are measured through ANOVA.

b- Interlaboratory studies : It is compared the reproducibility of routine methods to that of reference methods to decide whether or not centralised calibration provide equivalent laboratory bias distributions. In that case the information is general as the routine methods can be different with no relation to a well define analytical method. Recommendations of ISO 5725 are followed.

Characteristics of RMs for calibration

Adequate recommendation will be given to guarantee physicochemical quality of milk, sample preparation and batch homogeneity, preservation and shelf life, in particular with concern to the choice of the milk, milk and sample handling, chemical preservatives and sample containers.
Also indications for appropriate component arrangement and concentration range will be provided referring to optimisation of calibration and accuracy through specific designs with recombined (modified) milk samples (O. Leray, 1998, FAIR CE 1997-1999).

Assigning reference values

To limit the risk of systematic bias and get the agreement of all laboratories and parties they should not be established by a single laboratory but instead by all the laboratories of the concerned group.

The way to define reference values relates on whether or not matrix effects exist with the routine methods.

Where there is no matrix effect representativeness of calibration milk is of lower importance and focus is made only on physicochemical quality and concentration characteristics. Reference values are determined using the means of reference results of all the laboratories obtained in an interlaboratory study (Figure 1).

This is the same way also used in case of matrix effects when using milk materials well representative of the area (e.g. silo bulk milk) but choice must then be made on whether or not final calibration adjustments are locally required in laboratories with regard to laboratory biases observed. The assigned values are here used for pre-calibration (assessing slope, linearity, inter-correction fittings) whereas calibration is completed using one or more bulk milks representative of the area.

When using recombined (so-called modified) milk samples, greatest interest must be given in maintaining the native physicochemical quality of milk hence representativeness may not be reached. Through the matrix effect so-prepared calibration sample are not on the average line of the population. The assigned values are then obtained through a correction from the bias between the routine and the reference method with one or more bulk milks representative of the area. Calibration can be completed for individual labs (as above mentioned) if the range of local biases is too large for the purpose of milk testing.

Calibration

Recommendations for calibration operations are to follow the normal procedures of manufacturers and ISO 8196 in which centralised calibration is mentioned as a possible option. This is to 1- Check and where needed optimize instrument fittings (pre-calibration), 2- Adjust calibration, 3- Assign values for control samples.

Conclusion

Centralised calibration associated to collective determination of reference values for calibration is considered as an optimum combination to assure harmonisation of milk recording analytical data. Methodologies and technical tools have already been defined, experimented showing large efficiency. Such a combined system should serve ICAR countries to evolve towards easier and cheaper calibration systems and respond to forthcoming analytical demands of milk recording (for instance on-farm analysis).

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Interlaboratory reference system & centralised calibration

Pre-requisites & standard optimum procedures

Olivier Leray, Cecalait, france

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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.

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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

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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

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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		Sample mishandling ; stage of lactation ; species
			carboxylic acids (citrate, lactate)		Diet, feeding (season, region); species (metabolism)
				NPN in CP calibration	Diet, feeding (season, region); species (metabolism)

FT-MIR Full Spectrum ?

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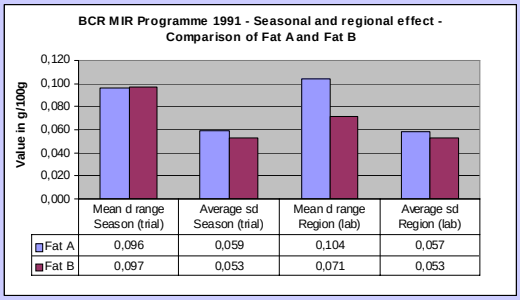

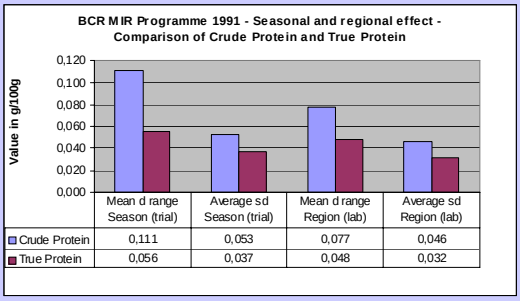

BCR MIR Prog. 1991:

15 European countries (labs)

8 trials on 1 year

2 bulk milks/trial/lab

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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)

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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

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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)*

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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

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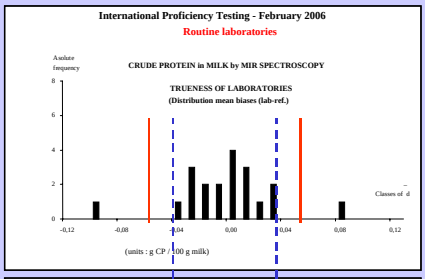
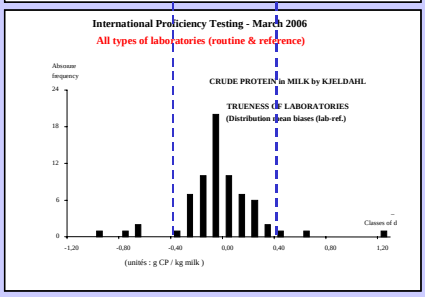
1a - Evaluation of current situation through PTs

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 ?

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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)

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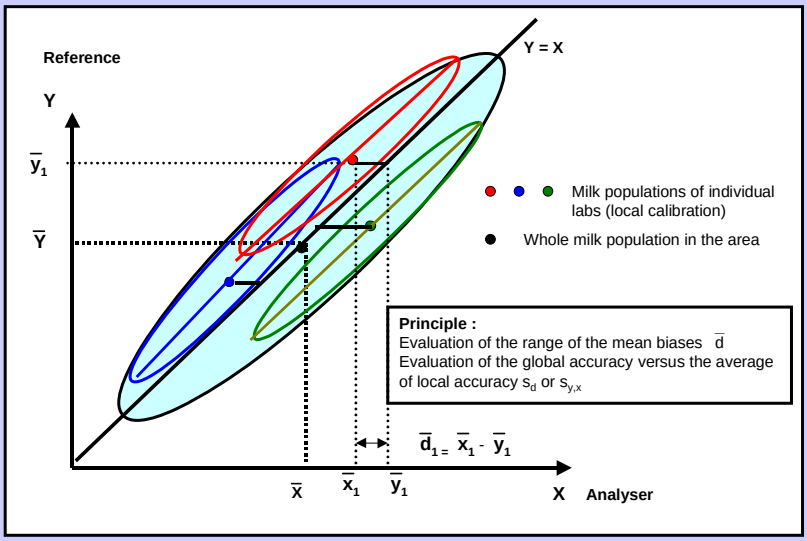
Principle of the evaluation of the regional effect and of the possible accuracy resulting of a centralised calibration



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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						
$LSD_{..}$					$LSD_{..}$	
$LSB_{..}$						$LSB_{..}$

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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*

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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)

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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

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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*

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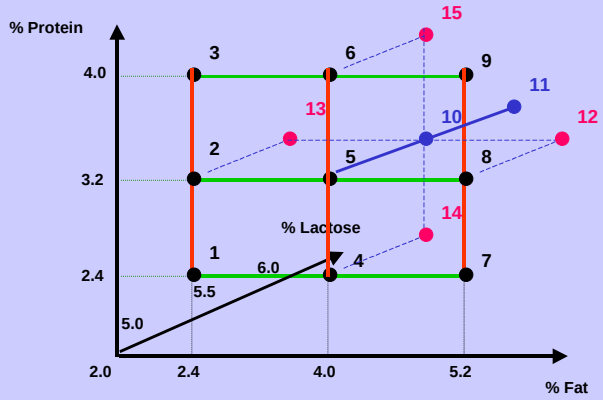
Example : Experimental design for MIR calibration
(recombined samples)



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

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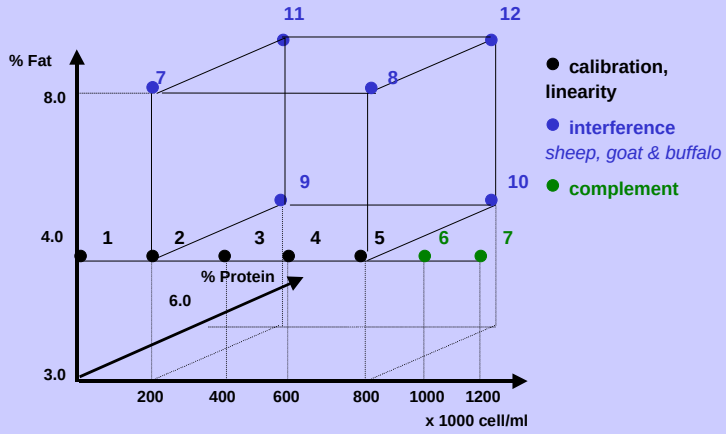
Example : Experimental design for SCC calibration
(recombined samples)



Cecalait, 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)

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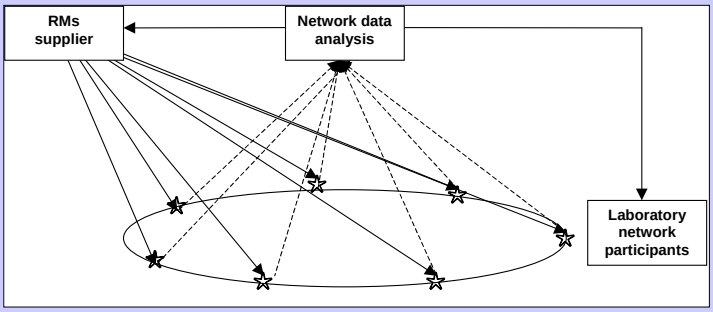
Central RM system for method with no matrix effect



General model : Numerous laboratories and samples ; robust reference

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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*

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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 $\overline{Y}_L$ **(ref)** & $\overline{X}_L$ **(rout)**

$Ref_C = Ref_R . (\overline{Y}_L / \overline{X}_L)$ or $Ref_C = Ref_R - (\overline{X}_L - \overline{Y}_L)$

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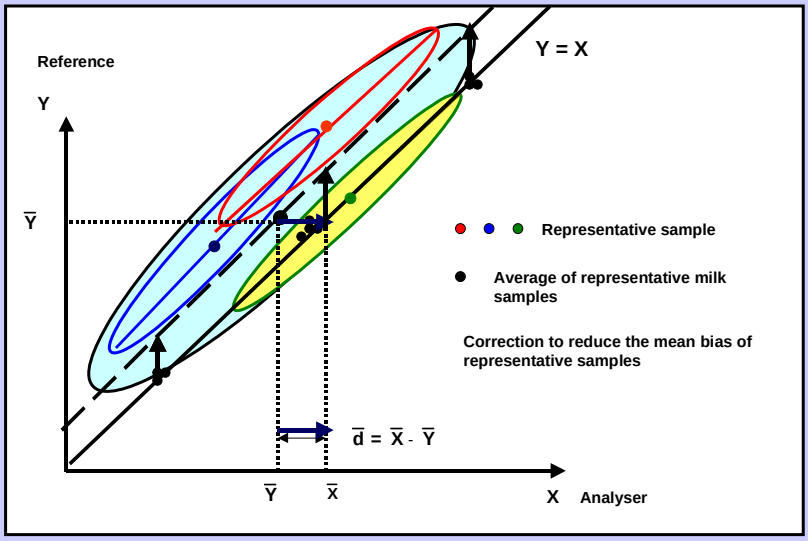
Centring theoretical values for centralised calibration



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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

⇒ Final correction:

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

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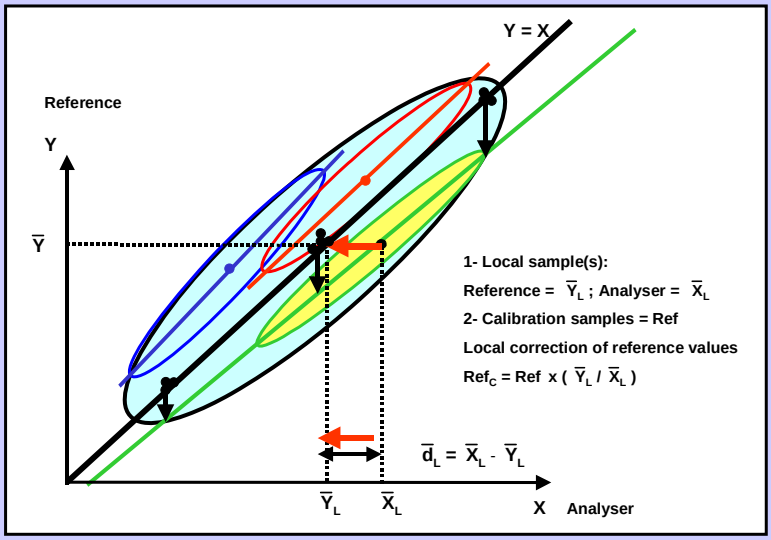
Local bias correction to reduce existing region effect



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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

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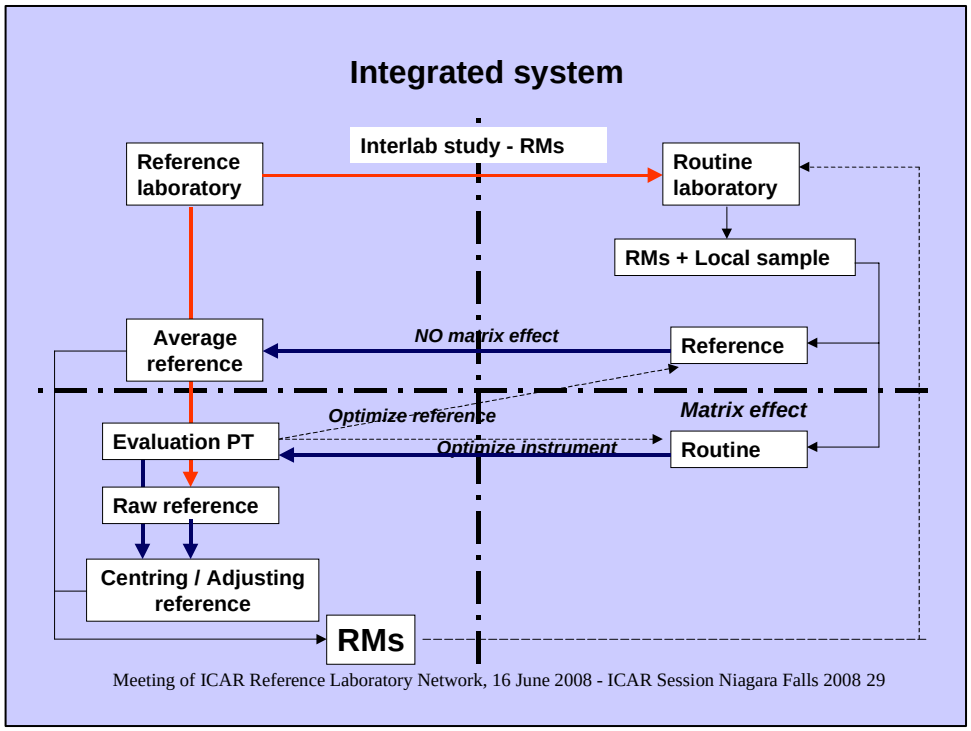
Integrated system
Interlab study - RMs
Reference laboratory
Routine laboratory
RMs + Local sample
Average reference
NO matrix effect
Reference
Optimize reference
Matrix effect
Evaluation PT
Optimize instrument
Routine
Raw reference
Centring / Adjusting reference
RMs
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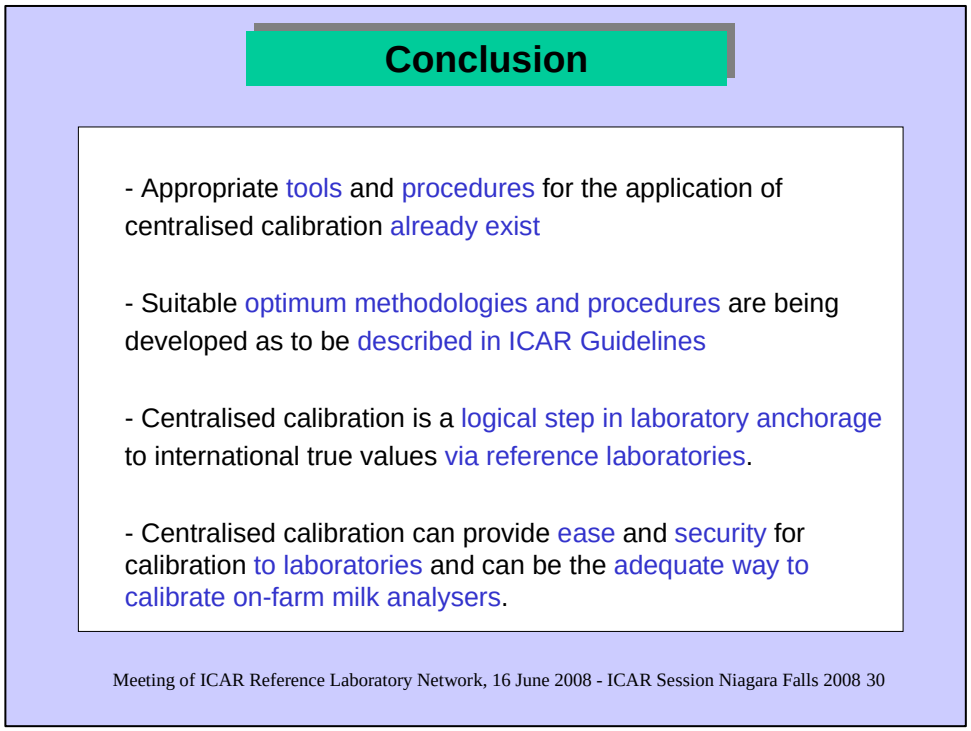
Conclusion
- Appropriate tools and procedures for the application of centralised calibration already exist
- Suitable optimum methodologies and procedures are being developed as to be described in ICAR Guidelines
- Centralised calibration is a logical step in laboratory anchorage to international true 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.
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The way to reference systems and centralised calibration for milk recording testing – Present status in Germany

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[1] MILCHPRUEFRING BAYERN e.V., Hochstatt 2, D-85283 Wolnzach, Germany

Abstract

In a globalizing world analytical results play a major role in free and fair trade. Global trade needs global validity of analytical results! This means that analytical results have to be equivalent worldwide at any place, at any time and despite what method has been used.

Some sources of error are affecting this analytical equivalence: Bad performance of reference methods and/or reference labs in characterizing (secondary) reference materials, insufficient reference materials in terms of imprecise target values and/or problems with shelf life, shipment etc. and failures in calibration of routine methods as well as information gaps and misinterpretations are hindering the optimal use of capabilities.

IDF has started a discussion about how to cope with these problems. In Bulletin 427/2008 a paper outlines the way towards a reference system for somatic cell counting as an example of how to come to a solution.

In this paper the author deals with the present status of implementing such a reference system in Germany. After a short description of the dairy sector in Germany and especially Bavaria, a picture is drawn of the DHI system and the laboratory work. Different aspects of QA in laboratories and the interlaboratory reference system in Germany are highlighted and it is shown, how the German system is interlinked with the ICAR Reference Laboratory System.

The author strongly pleads for cooperation between analysts on all levels. ICAR should join the IDF activities and assist in creating an international structure for reference systems. Centralized calibration procedures could be one of the tools to step forward.

Keywords: *analytical reference systems, centralised calibration, equivalence of analytical results, dairy sector in Germany, interlaboratory reference system;*

remarks on reference systems

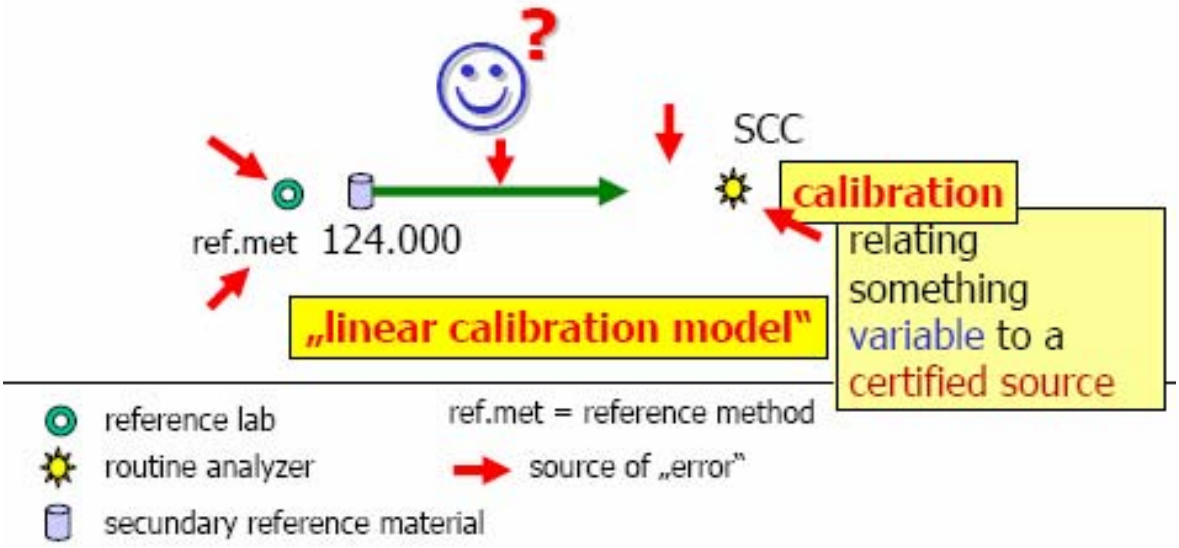
- global trade needs global validity of analytical results!
- validity means equivalence
  - worldwide at any place
  - at any point in time
  - despite what method is used

➔ anywhere – anytime – anyhow

sources of „errors" in equivalence

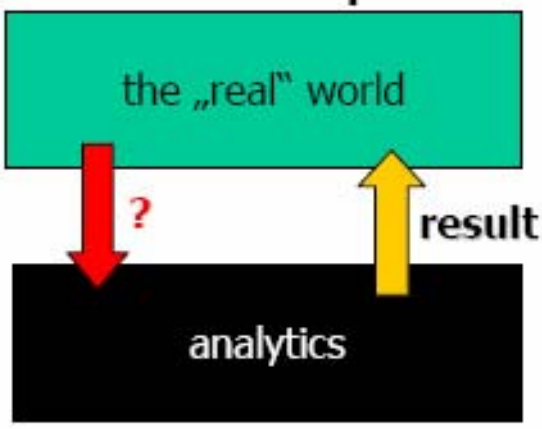
- reference method
- reference lab
- SRM or CRM
  - characterization
  - „quality" (shelf life, shipment, ...)
- calibration procedure
- routine method
- information failures (missing, comparability)

calibration – the traditional way of life



the analytical problem

what about interpretation?

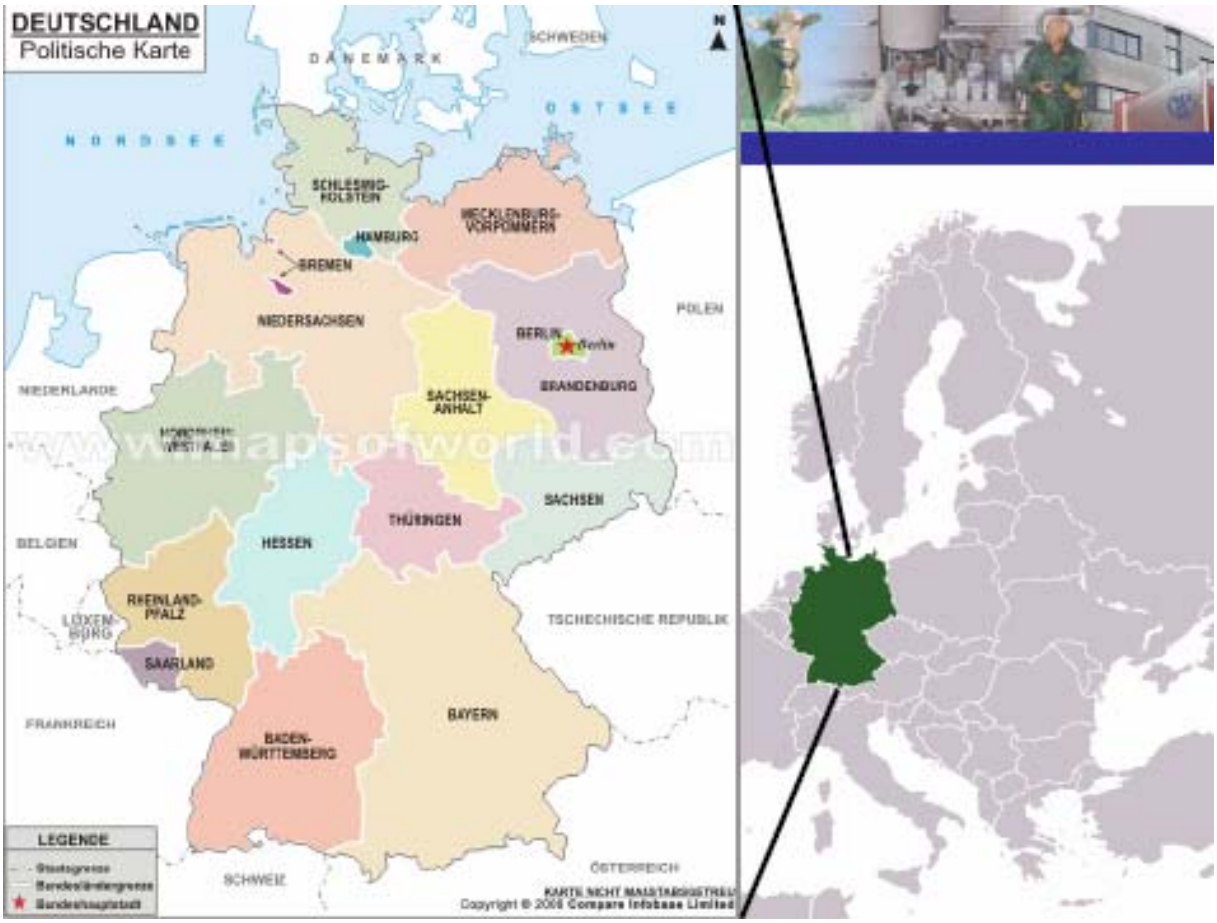


differences?
➔ who is right?

claim
➔ there is only one truth!

do we need centralised calibration?

- we need calibration, because we have to use routine methods
  - high throughput
  - high performance (precision characteristics)
  - data availability and handling
  - low labour, low costs
- traditional linear calibration schemes have to be interlinked to reduce equivalence failures



situation in Germany (12.2007)

- 65.850 farms under DHI (of 95.870)
- 3.514.000 cows under DHI (of 4.730.000)
- ~35 mio. DHI samples
- 19 labs, ~330 staff
- ~50 kombi-analyzers

sources of „errors" in equivalence

interlaboratory reference system

- reference method
- reference lab
- SRM or CRM
  - characterization
  - „quality" (shelf life, shipment, ...)

lab QA

- calibration procedure
- routine method
- information failures (missing, comparability)

sources of „error

interlaboratory reference system

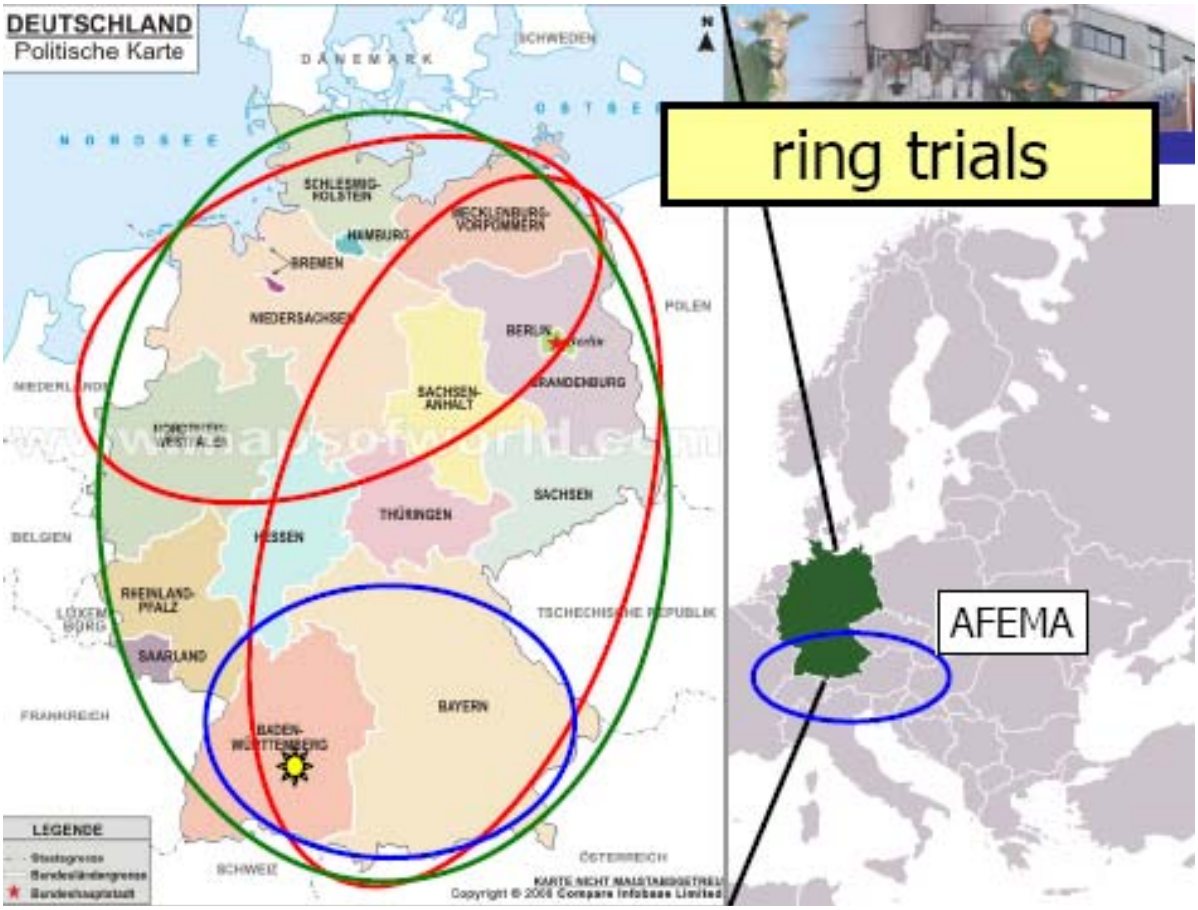
- reference method
- reference lab
- SRM or CRM
  - characterization
  - „quality" (shelf life

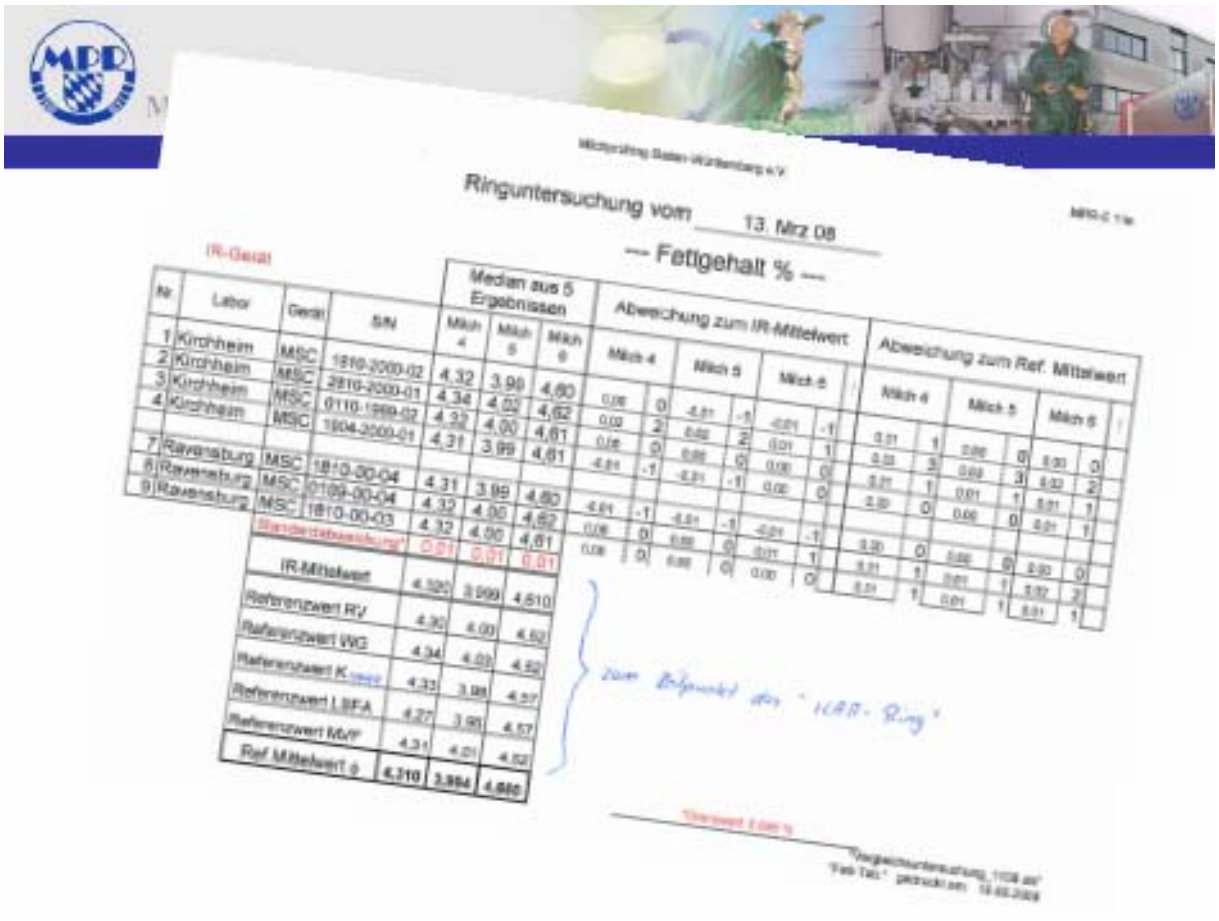
fat	protein	SCC	urea
√	√	√	√
~10	~10	?	√
3-5	3-5	2	?

QSE – deep frozen RM
(8 ref.labs – very long shelf life)

lab QA

- calibration procedure
- routine method
- information failures (missing, comparability)





outlook

- Analytical people are more and more aware that equivalency means cooperation on all levels – locally – nationally – internationally!
- International structures for implementation of reference systems are missing so far. What can ICAR do?
- Joint new work item with IDF on reference systems! Centralised calibration is one issue...

Reference system and centralized calibration for milk recording testing in Argentina

Lic Roberto Castañeda

INTI Lácteos. Buenos Aires, Argentina.

Introduction

Milk production in Argentina was over 10 billion liters in 2006. This figure positions the country in the 11th place in the ranking of world milk producers, and in the 2nd as regards Latin America. There are 2.5 million dairy cows, most of them pertaining to *Holando-Argentina* breed, producing approximately 4000 liters of milk/cow/year. The Argentine dairy industry is geographically distributed all over the country. Five provinces making up the so-called Pampeana Region produce 94% of the milk in a surface area of 800,000 square kilometers. The country has 14,000 dairy farms and 1100 dairies of different sizes where milk products are manufactured. This milk is mainly used in the production of cheese (45%); milk powder (24%); pasteurized and sterilized fluid milk (19%) and other products.

The "*Holando- Argentina"* breed was introduced into Argentina from Holland in 1880. These cows are medium sized with the height of 1.40 to 1.5 meters, having a large barrel allowing them to have a high intake of forage. In 1944, breeders create an organization to promote the breed and to provide necessary technical support named *Holando- Argentina* Breeders Association, ACHA. In 1981, the government (Department of Agriculture) delegate by law the "official dairy herd improvement" system in ACHA. The breeder association is a full member of ICAR in 1991 and subscribed an agreement with INTI, the National Institute of Industrial Technology in 2003, for the creation of a technical assistance and control laboratory network that began to work the follow year, committing DHI laboratories to participate in proficiency testing schemes under REDELAC, the network of INTI.

Milk control in Argentina

Since early in the 20th Century milk producers in the Argentine Republic started to control their cows' production with the purpose of improving cattle quality. Nowadays we have: 2.000 dairy farms in "official milk control", 510.000 cows under this system, 11 DHI laboratories that analyze the composition of the milk and a reference national laboratory that control the performance of DHI laboratories. Tests carried out include milk fat and protein content, and somatic cell count.

Testing laboratories for milk recording

There are currently 11 laboratories conducting tests for official milk control in different provinces. Most of them are private and/or provincial laboratories, independent of producers or of the industry, supplying services to the milk chain, essentially as regards milk control, milk payment according to quality standards and other process control tests. Testing laboratories are distributed in different provinces according to the list in Table 1.
On a monthly basis, results obtained at these laboratories participate in control schemes with the National Reference Laboratory namely INTI-LÁCTEOS who has, jointly with ACHA, the mission to supervise the laboratories supplying services to Official Milk Control Entities, as well as to provide technical support in equipment calibration and training the corresponding human resources.

Table 1. List of milk testing laboratories operating for milk recording in Argentina

NAME	CITY	PROVINCE	BELOGS TO
ALECOL .	Esperanza.	Santa Fe.	Milk producers
CERET	General Pico	La Pampa	Provincial state
FUNESIL	Villa Maria	Cordoba	Private
INSULAB	Venado Tuerto	Santa Fe	Private
LABROLAC	Las Varillas	Cordoba	Milk producers
LABVIMA	Trenque Lauquen	Buenos Aires	Private
LABVIMA	Villa Maria	Cordoba	Private
LACLE	Buenos Aires	Capital Federal	Private
LEVER	Paraná	Entre Rios	Provincial
MATCO	Lujan	Buenos Aires	Private
SANCOR	Sunchales	Santa Fe	Dairy Industry

National reference laboratory

INTI LÁCTEOS is the laboratory appointed by ACHA as the reference laboratory, with vast experience in technical assistance to milk labs; it is also the supplier of interlaboratory trials and reference materials. In turn, and complying with ICAR instructions, ACHA has requested the inclusion of INTI Lácteos as the national reference laboratory (NRL) for Argentina in ICAR laboratory network.

INTI LACTEOS is the Technological Research Center for the Milk Industry and was created in 1968. It is one of the nearly 40 INTI centers, the National Institute of Industrial Technology, a decentralized entity depending upon the Argentine Ministry of Economy. INTI, among many other responsibilities, is the National Metrology Institute in Argentina.

Seventy five professionals and technicians work at INTI LÁCTEOS, providing consultancy and technical support to all links in the milk chain, and among them, to testing laboratories. The center is headquartered in the city of San Martin, in the province of Buenos Aires and in the city of Rafaela, in the province of Santa Fe. Its scope includes training, assistance, development, innovation and testing activities. INTI LACTEOS has laboratories for milk quality, physicochemical testing, microbiology, residues and contaminants, sensory evaluation, and others.

In compliance with ICAR requirements regarding the mandatory character of maintaining certified quality systems, INTI Lácteos labs in Buenos Aires and Rafaela conduct analytical assessments, organize proficiency test programs and supply reference milk material pursuant to ISO 17025, ISO 43, ILAC G13, and ISO 34 systems, certified by the official Argentine Accreditation Body (OAA) and the National Accreditation Entity of Spain (ENAC).

Since 1991 INTI LÁCTEOS has also been the reference laboratory for REDELAC (www.redelac.gov.ar), a network of Argentine milk laboratories developed by INTI itself, whose purpose is to provide such laboratories with the tools to maintain their technical competence. Milk industry laboratories are included in this network; there are many of them with high technical competence, and some of food laboratories in general. INTI LÁCTEOS maintains a wide suitability testing program for different milk matrixes that has been accredited by ENAC since 10/15/04, through Certificate 001/PPI001. It has also developed a centralized calibration system for milk analysis instruments, called SICECAL, currently in the certification process under ISO 34 Standard.

Assistance and external control of milk testing laboratories

Technical assistance and control of milk testing laboratories result from a wide experience in this field, where work has been done since 1991 in order to obtain homogeneity in results and maintain

metrological traceability between the testing laboratory, the national reference laboratory and international labs.

Assistance consists in training actions, both in analytical tests subjects and in quality assurance subjects. Laboratory control is carried out through a scheme based on 1) centralized calibration, 2) control of performance of laboratories and 3) an evaluation of the laboratories by an ACHA-INTI committee to ascertain its performance and to set the adequate corrective actions if required.

1- Centralized calibration system SICECAL:
SICECAL is a system of preparation, analysis and delivery of reference materials in dairy matrix for calibration and control equipment. It is a widely used tool in Argentina to calibrate different types of analyzers used in milk laboratories. It consists in sending monthly standard samples for

- calibration of infrared analyzers (fat, proteins, totals solids, lactose, ash)
- adjustment of fluoro-opto-electronic equipment for somatic cell count
- calibration of milk cryoscopes
- others

The use of these Reference Materials is not mandatory, and this is so since there are big laboratories that prepare their own materials.

This Reference Materials are produced in INTI Lácteos in Rafaela according the requirements of the guide ISO 35. For calibration or IR equipment, 11 and 5 samples of raw milk are sent in ***the first week*** of the month. Composition: fat: 2.50 to 5.00 g/100 ml, protein: 3,00 to 3.60 g/100 ml, lactose: 4.60 to 5.00, ash: 0.68 to 0.82 and dry matter content: 11.80 to 13.80. Milk composition is informed with the pertinent uncertainty. Participants receive a delivery schedule early each year.

For adjustment of somatic cell equipments, 3 samples of raw milk are sent in the first week of the “pair” months. Composition: “low” somatic cells counting (170.000 cel/ml); “medium” (430.000 cel/ml); and “high” (700.000 cel/ml).

Samples are prepared with mixed raw milk. The reference value is obtained by IDF reference methods in quadruplicate. There are checks of the reference value (named “previous SICECAL”) where the laboratory test the value in four (4) IR-equipment or SC-equipment in other recognized laboratories. Test of homogeneity and stability are performing according to the requirements in guide ISO 35. With these samples, laboratories calibrate, re-calibrate, verify or adjust testing equipment.

2- Performance assessment of DHI laboratories:
The control of performance of DHI laboratories is carried out through two types of actions.

- *Monthly check* of results of laboratories. Every second Tuesday of the month, (one week after centralized calibration), laboratories receive one sample to analyze fat, total proteins and somatic cells count by their routine methods. They are obliged to send results in time to INTI Lácteos.
- *An Bi-annual interlaboratory trial*. Each six month, the laboratories receive 10 samples to analyze fat, total proteins and SCC. They must submit results in time to INTI Lácteos.

In the *monthly check* DHI laboratories receive one blind sample for each parameter to check, to be analyzed in a period of time. The comparison of results with INTI Lácteos permit assures the suitability of the equipment to conduct milk control tests. Samples are prepared with mixed raw milk. Composition: 2.5-4 % of fat, 2.8-3.5 % total proteins, 100.000-700.000 SCC, and others. Test of homogeneity and stability are performing according ISO 13528 standard. Usually, as these laboratories also analyze samples for milk payment purpose, they also receive additional samples to check the results of other milk quality parameters (antibiotic residues, bacteria total count and freezing point). It is interesting to remark that logistics for sending these samples is not a minor topic, since samples have to arrive at laboratories in time and good state of preservation.

Results of laboratories are compared against the reference value obtained by INTI Lácteos in Buenos Aires by using IDF reference methods, and applying an ISO 17025 quality system accredited by the OAA. The reference value must be not statistically different of the robust media (26 laboratories nowadays). If

yes, the NRL studied the reason and decide which reference will be use. Next, a results report is issued where it is shown whether results obtained for each test are comparable to results obtained by NRL, the performance of the latest 12 month of the laboratory and a comparison of the laboratory with the other laboratories participating in the PT scheme.

In the *bi-annual interlaboratory trial*, laboratories must participate in a proficiency test where 10 samples with variable percentages of fat, protein, lactose, total solids content and somatic cell count are sent. This inter-comparison scheme is improved under an ISO 43 / ILAC G13 quality system accredited by ENAC. The NRL send 10 different samples for each component. They are prepared with raw milk as IDF Standard 141:2000 by separation and recombination of components. The composition is: range of 2.5-4 % for fat; 2.5-3.5 % for total proteins; and 100.000-700.000 for SCC. Test of homogeneity and stability are performing according ISO 13528. The reference value is obtained by consensus of all laboratories, calculating robust media. INTI Lácteos analyze also the samples by IDF reference methods, in duplicate, to assure results.

3- Evaluation of results:
The results of these reports are analyzed by an INTI-ACHA Advisory Committee created within the framework of the technological linkage agreement subscribed by both institutions. This advisory committee hold meeting every two month and decides the actions to follow according the evaluation of each laboratory.

Conclusion

The reference system for milk recording testing in Argentina is based on the action of a national reference laboratory and DHI dairy laboratories, which interchange information, technical assistance and control mechanisms. The characteristics of our country and our milk permit a centralized calibration of testing equipment and a frequent control of milk recording testing laboratories. At a time, the NRL check your own performance by means of PT schemes with international institutions. These metrological scheme permit Argentina maintain a good traceability between laboratories and international institutions by means of inter-comparisons. This characteristics show a metrological system for milk measurements according the importance of the argentine dairy industry.

National Institute of Industrial Technology, INTI.
Dairy Industry Technological Research Centre. INTI LACTEOS
Buenos Aires. Argentina.

"Reference system and centralized calibration for milk recording testing in Argentina."

Roberto Castañeda
ICAR Reference Laboratory Network.
Niagara Falls. USA. June 16, 2008

Dairy sector in Argentina

- **Milk production in Argentina: 10 billion liters in 2006**
- **11° place in the ranking of milk world producer.**
- **2nd place in Latin America.**
- **2.5 million dairy cows.**
- **Most of them "holando argentina" breed.**
- **94.5 % of the milk produced is in Pampeana Region (800.000 km ²)**
- **14.000 dairy farms**
- **1.100 dairies of different sizes.**

In 1880 "*Holando Argentina*" breed was introduced from Holland.

The *Holando-Argentina* cows are medium sized with the height of 1.40 to 1.5 meters. These animal have a large barrel allowing them to have a high intake of forage.

In 1944, breeders create an organization to promote the breed and to provide necessary technical support named *Holando- Argentina* Breeders Association, ACHA.

In 1981, de government (Department of Agriculture) delegate by law the "official milk control" system in ACHA.

In 1991 is a full member of ICAR.

In 2003 ACHA subscribed an agreement with INTI for the creation of a technical assistance and control laboratory network.

Nowadays we have:

2.000 dairy farms in "official milk control"

510.000 cows under this system

11 DHI laboratories that analyze the composition of the milk.

A reference national laboratory that control the performance of DHI laboratories.


DHI Laboratories in Argentina:
ALECOL. Esperanza. SANTA FE.
CERET. Gral. Pico. LA PAMPA
FUNESIL. Villa Maria. CORDOBA
INSULAB. Venado Tuerto. SANTA FE.
LABROLAC. Las Varillas. CORDOBA.
LABVIMA. Villa Maria. CORDOBA.
LABVIMA. Trenque Lauquen. B AIRES
LACLE. Capital Federal.
PRIVATE LABS
PROVINCIAL DAIRY LABS
INDUSTRIES
PRODUCERS
o Official milk control.
o Milk payment purpose
o Process control purpose
o Final product control
INTI
Antofagasta
Salta
Asunción
São Paulo
Rio de Janeiro
San Miguel de Tucumán
Córdoba
Santa Fe
Rosario
Mendoza
Santiago
Buenos Aires
Montevideo
Concepción
Mar del Plata
Bahía Blanca
Valdivia
Comodoro Rivadavia
Florianópolis
Porto Alegre
Stanley


INTI Lácteos
Dairy Industry Technological Research Centre.
2 work places: in Buenos Aires and Rafaela.
75 professionals and technicians.
Laboratories for milk quality, physicochemical testing, microbiology,
INTI LACTEOS:
National Reference Laboratory
INTI
Rafaela
Aires
Antofagasta
Salta
Asunción
São Paulo
Rio de Janeiro
San Miguel de Tucumán
Córdoba
Santa Fe
Rosario
Mendoza
Santiago
Montevideo
Concepción
Mar del Plata
Bahía Blanca
Valdivia
Comodoro Rivadavia
Florianópolis
Porto Alegre
Stanley

INTI LACTEOS: National Reference Laboratory

INTI Lácteos is one of the 35 centers of INTI, the Argentine Institute of Metrology.

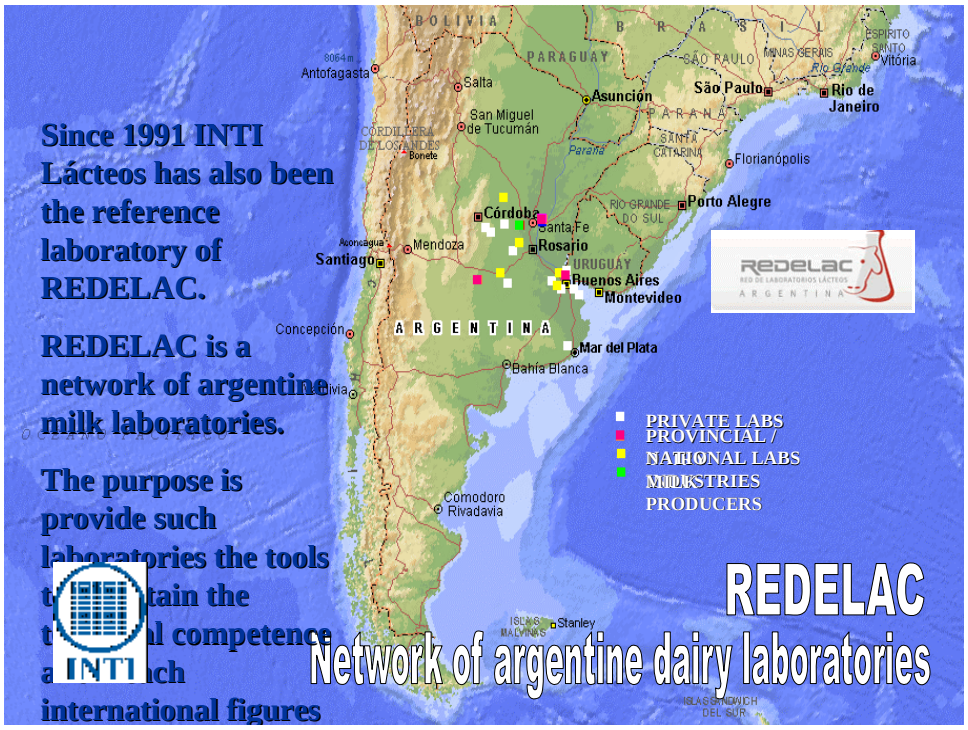
Reference National Laboratory for "milk control" systems (ACHA) and for milk payment purposes (Agriculture Ministry).

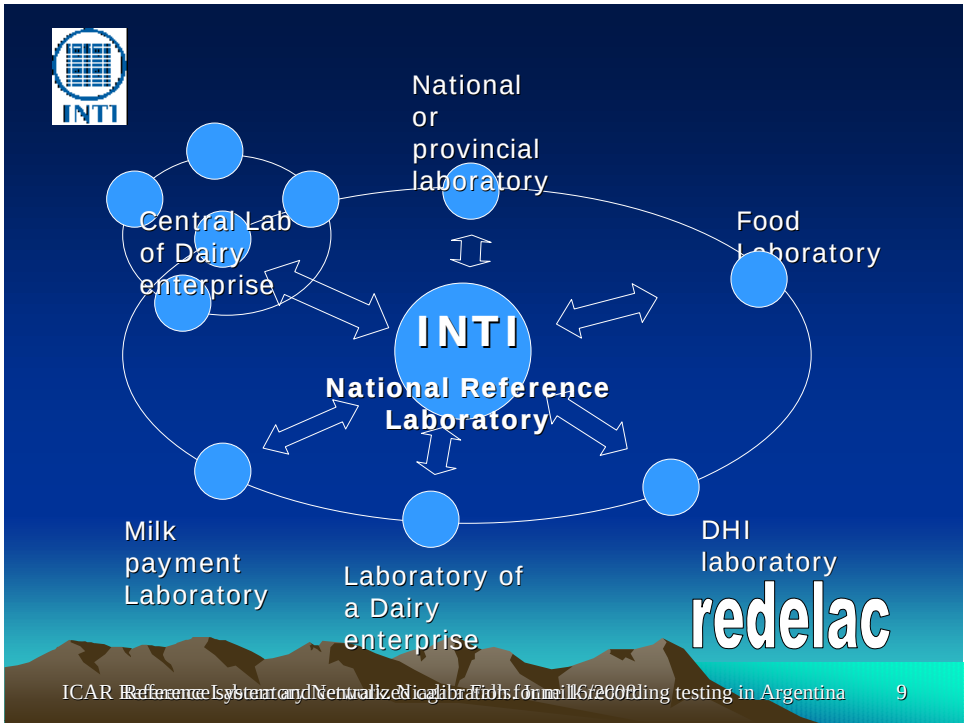
Quality system according ISO 17025 accreditated by OAA (National Organization of Accreditation.)

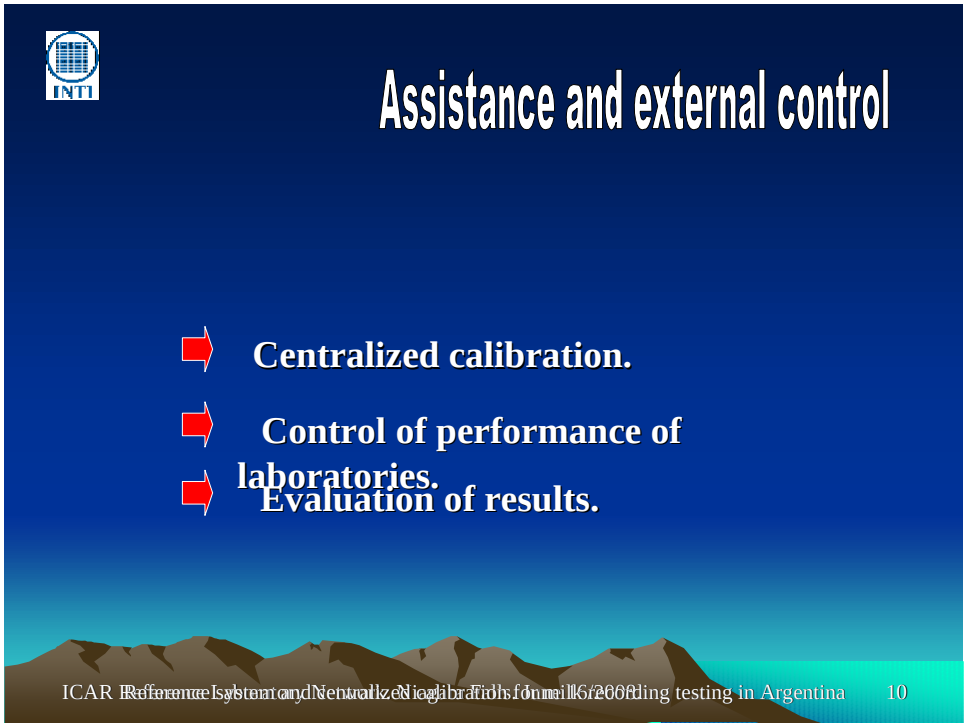
PT provider in Argentina and the south american region.

Quality system according ISO 43/Guide ILAC G 13 accreditated by ENAC (Spanish Organization of Accreditation).

ICAR Reference System and Centralized Calibrations for milk recording testing in Argentina 7




National or provincial laboratory
Central Lab of Dairy enterprise
Food Laboratory
INTI
National Reference Laboratory
Milk payment Laboratory
Laboratory of a Dairy enterprise
DHI laboratory
redelac
ICAR Reference system and Neutralized calibrations for milk recording testing in Argentina 9


Assistance and external control
Centralized calibration.
Control of performance of laboratories.
Evaluation of results.
ICAR Reference system and Neutralized calibrations for milk recording testing in Argentina 10

SICECAL is a system of preparation, analysis and delivery of reference materials in dairy matrix for calibration and control equipment. It is a widely used tool in Argentina.

- **calibration of infrared analyzers** (fat, proteins, totals solids, lactose, ash)
- **adjustment of fluoro-opto-electronic equipment** for somatic cell count

This Reference Materials are produced according the requirements in guide ISO 35.

11 and 5 samples of raw milk are sent in ***the first week*** of the month. Composition: fat: 2.50 to 5.00 g/100 ml, protein: 3,00 to 3.60 g/100 ml, lactose: 4.60 to 5.00, ash: 0.68 to 0.82 and dry matter content: 11.80 to 13.80.

3 samples of raw milk are sent in the first week of the "pair" months. Composition: somatic cells counting: low (170.000 cel/ml); medium (430.000 cel/ml); and high (1.000.000 cel/ml)

centralized calibration (Sicecal)

With these samples, laboratories calibrate, re-calibrate, verify or adjust testing equipment.

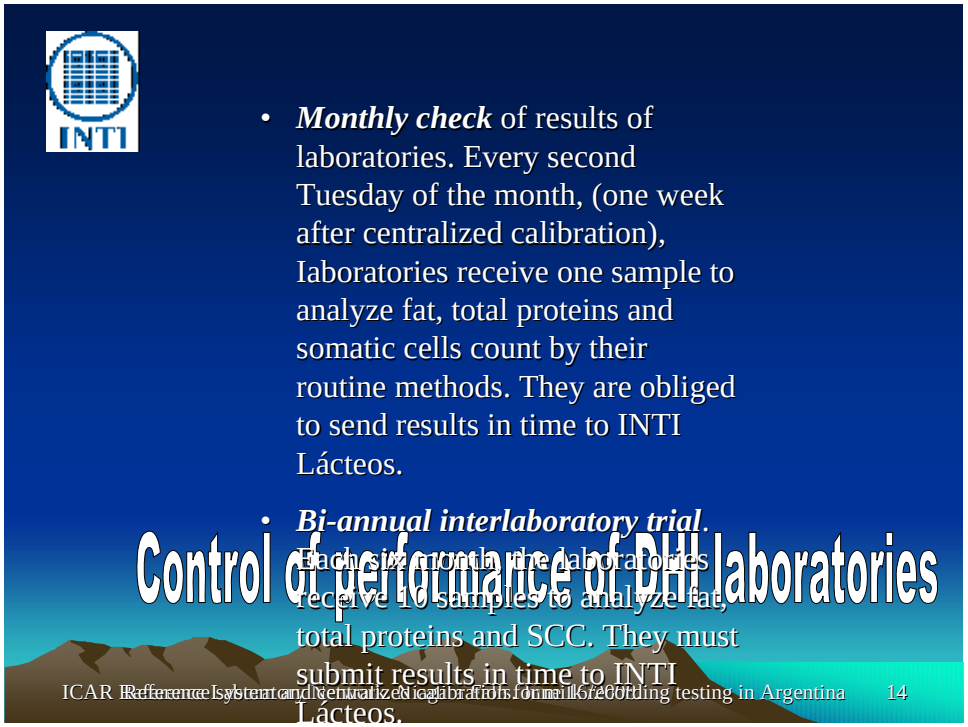
Samples are prepared with mixed raw milk.

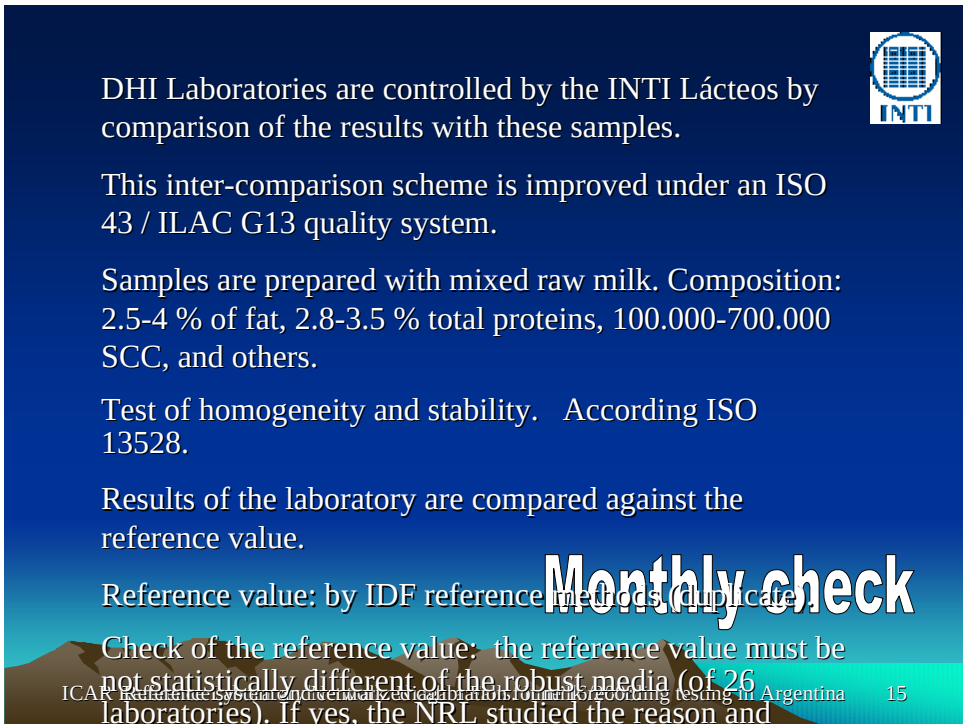
Reference value: by IDF reference methods (quadruplicate).

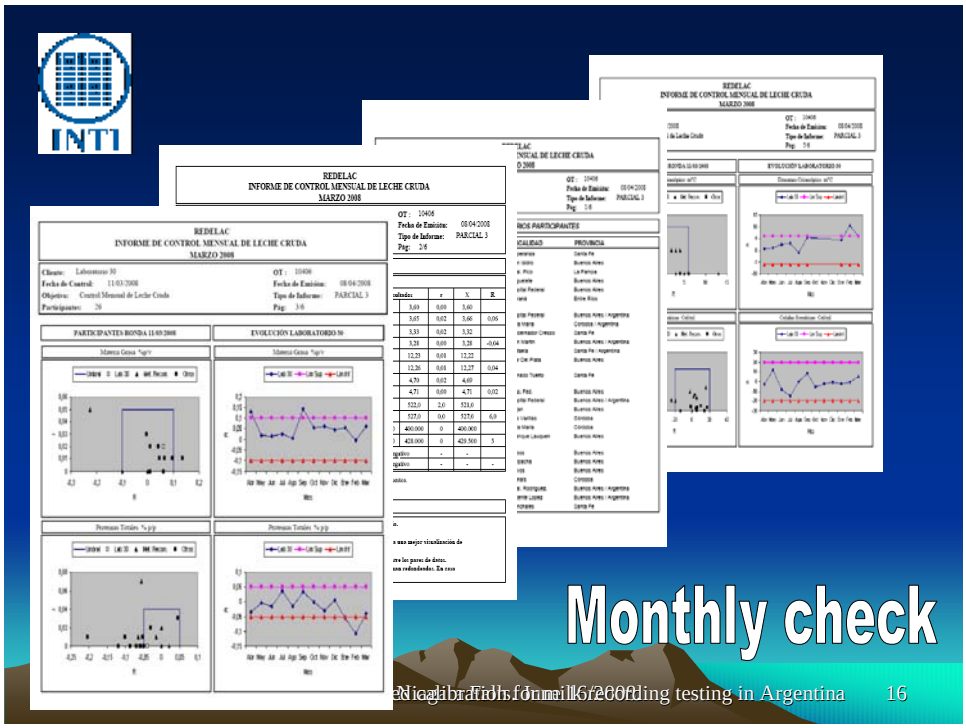
Check of the reference value (previous SICECAL): in 4 IR-equipment or SC-equipment in recognized laboratories.

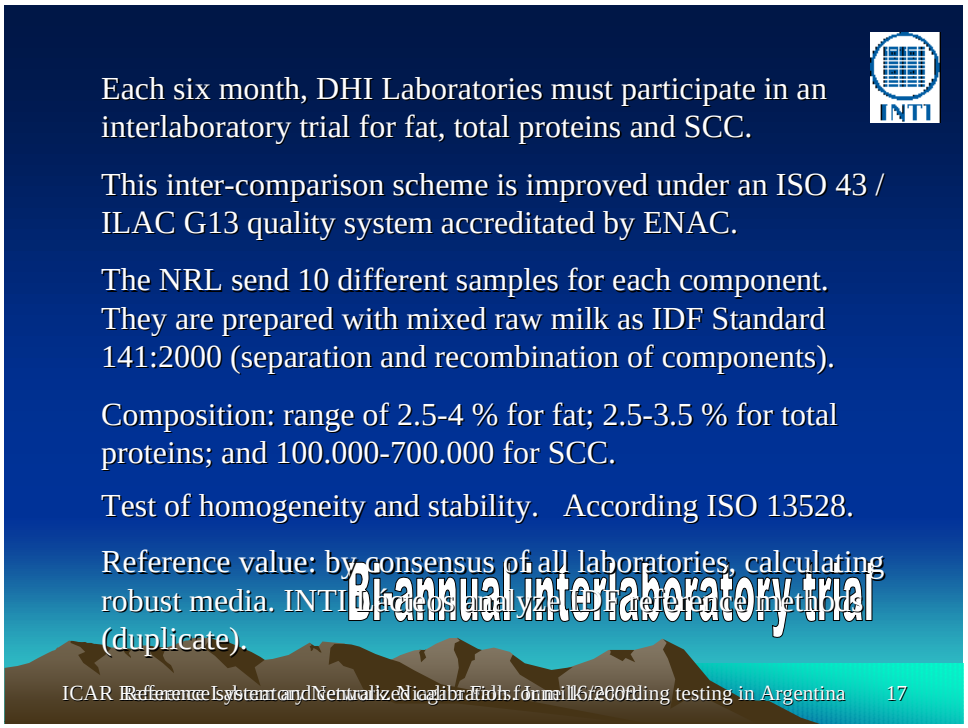
Test of homogeneity and stability. According to the requirements in guide ISO 35 and the document "Statistical Aspects of the certification of chemical batch SRMs of the NIST.

centralized calibration


INTI
• Monthly check of results of laboratories. Every second Tuesday of the month, (one week after centralized calibration), laboratories receive one sample to analyze fat, total proteins and somatic cells count by their routine methods. They are obliged to send results in time to INTI Lácteos.
• Bi-annual interlaboratory trial. Each six month the laboratories receive 10 samples to analyze fat, total proteins and SCC. They must submit results in time to INTI Lácteos.
Control of performance of DHI laboratories
ICAR Reference system and centralized calibration for milk recording testing in Argentina 14


INTI
DHI Laboratories are controlled by the INTI Lácteos by comparison of the results with these samples.
This inter-comparison scheme is improved under an ISO 43 / ILAC G13 quality system.
Samples are prepared with mixed raw milk. Composition: 2.5-4 % of fat, 2.8-3.5 % total proteins, 100.000-700.000 SCC, and others.
Test of homogeneity and stability. According ISO 13528.
Results of the laboratory are compared against the reference value.
Reference value: by IDF reference methods (duplicate).
Check of the reference value: the reference value must be not statistically different of the robust media (of 26 laboratories). If yes, the NRL studied the reason and
Monthly check
ICAR Reference system and centralized calibration for milk recording testing in Argentina 15

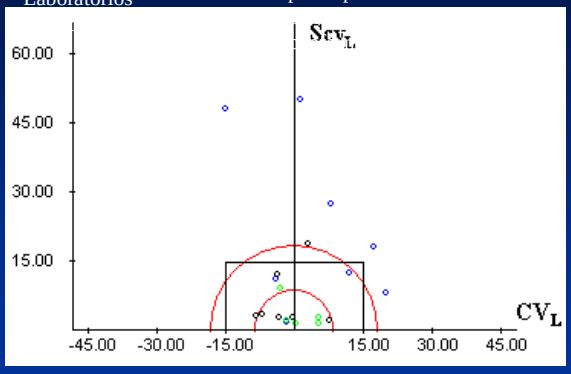

REDELAC
INFORME DE CONTROL MENSUAL DE LECHE CRUDA
MARZO 2008
Monthly check
ICAR Reference System and Centralized Calibration for milk recording testing in Argentina 16


Each six month, DHI Laboratories must participate in an interlaboratory trial for fat, total proteins and SCC.
This inter-comparison scheme is improved under an ISO 43 / ILAC G13 quality system accreditated by ENAC.
The NRL send 10 different samples for each component. They are prepared with mixed raw milk as IDF Standard 141:2000 (separation and recombination of components).
Composition: range of 2.5-4 % for fat; 2.5-3.5 % for total proteins; and 100.000-700.000 for SCC.
Test of homogeneity and stability. According ISO 13528.
Reference value: by consensus of all laboratories, calculating robust media. INTI analyzes analyze IDF reference methods (duplicate).
Bi annual interlaboratory trial
ICAR Reference System and Centralized Calibration for milk recording testing in Argentina 17

RECUENTO DE CELULAS SOMATICAS TOTALES

20 Laboratorios

70% de los Laboratorios participantes



70 % de los laboratorios bajo el **primer semicírculo**.
45% de los laboratorios bajo el **segundo semicírculo**.
Los laboratorios fueron diferenciados de la siguiente manera, según el método empleado para el análisis:

Microscopía Optica Fossoma Somacount

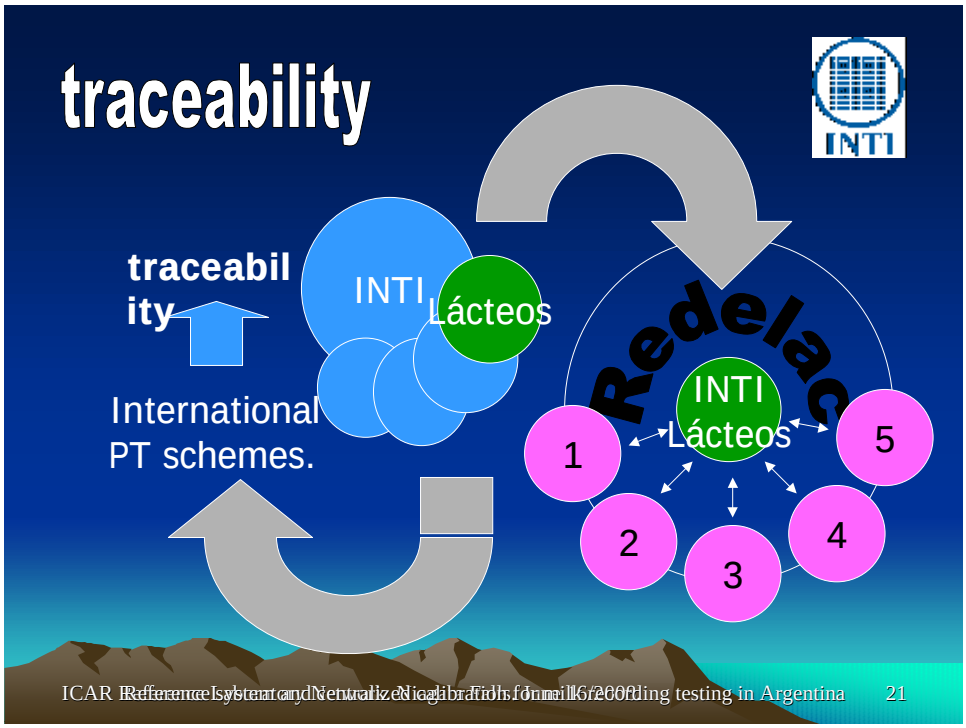
Límites de la superficie de conformidad: **CVL**=± 15 %
ScvL= 15 %

The results of these reports are analyzed by an INTI-ACHA Advisory Committee created within the framework of the technological linkage agreement subscribed by both institutions.

This advisory committee hold meeting every two month and decides the actions to follow according the evaluation of each laboratory.

Evaluation of results


These metrological scheme permit Argentina maintain a good traceability between laboratories and international institutions by means of inter-comparisons.
traceability
ICAR Reference system and centralized calibration for milk recording testing in Argentina 20


traceability
traceabil ity
International PT schemes.
INTI
Lácteos
Redelac
INTI
Lácteos
1
2
3
4
5
ICAR Reference system and centralized calibration for milk recording testing in Argentina 21

The reference system for milk recording testing in Argentina is based on the action of a national reference laboratory and dairy laboratories, which interchange information, technical assistance and control mechanisms.

The characteristics of our country and our milk permit a centralized calibration and a frequent control for milk recording testing.

In this way, Argentina maintains a good traceability scheme between laboratories and international institutions by means of inter-comparisons.

conclusions

This characteristics shows a metrological system for milk measurements according the importance of the argentine dairy industry.

National Institute of Industrial Technology, INTI.
Dairy Industry Technological Research Centre. INTI LACTEOS
Buenos Aires. Argentina.

Reference system and centralized calibration for milk recording testing in Argentina

castaned@inti.gov.ar

thank you ! muchas gracias...

Reference system and centralised calibration for milk (payment) testing

David Barbano

Cornell University, Department of Food Science, Ithaca, NY 14853, USA

Abstract

A modified milk calibration set has been developed for use in a network of payment testing laboratories in the US. The ser of calibration samples consist of 14 samples produced with an orthogonal matrix of composition with respect to variation in fat, protein, and lactose. The range of fat content is from 0.2 to 5.8%, true protein from 2 to 4.3%, and anhydrous lactose from 3.9 to 5.2%. The modified milk calibration samples are produced 12 times per year and serve as a proficiency test for the reference chemistry methods performed in all the laboratories and a set of calibration samples for infrared milk analyzers. These samples are used to set slope and intercept of the intercorrected mid-IR signal.

The modified milk calibration samples serve three purposes. First, each month the testing of these samples provides a proficiency test of the fat by ether extraction, the true protein by Kjeldahl, the anhydrous lactose by enzymatic, and total solids by oven drying methods. The orthogonal matrix of composition of the set of samples provides some interesting diagnostic and trouble shooting opportunities that are used to improve the performance of the laboratories that run the chemistry methods. The performance of individual laboratories and the group of laboratories for the chemistry methods has been improved. Second, the all-laboratory mean with outliers removed is used to create a fat, protein, and lactose reference value for each sample. Third, the samples are used for 1 month to set the slope and intercepts for each instrument. Because of the orthogonal matrix of composition, the data can be used to evaluate the linearity and intercorrection response of each instrument. These evaluation calculations and protocols are built into a software package we have written called IR-QC. Instrument Calibration Performance has been improved by using the modified milk calibration samples and all-lab mean reference values. The standard deviation of the difference between reference chemistry and instrument values on all components is < 0.015% and often < 0.01% using a traditional filter based calibration approach. The size of the 95% confidence interval around the slope of the regression line has been reduced greatly by the use of the modified milk calibration samples, compared to the performance that is achieved by using raw milks from individual farms for calibration. This is due to the homogeneity of the matrix of the modified milks and elimination of the influence of high leverage samples from the calibration set.

The network of laboratories does monthly pre-calibration performance evaluations of instrument performance. Homogenizer performance is monitored by a central laboratory at Cornell University using laser light scattering particle size analysis. Homogenizers that have failed the homogenization performance evaluation by particle size analysis are inspected by microscopic evaluation to determine the cause of failure.

In our research we have developed an optimized set of traditional “virtual” sample and reference filter wavelengths for use in FTIR instruments and we are in the process of publication of that information. We have also made a quantitative determination of the impact of variation in fatty chain length and unsaturation on Fat B and Fat A on absorbance at sample and reference wavelengths with a model sample system. That work is complete and in the process of publication. We continue to work toward the goal of improving the accuracy of the infrared milk testing to achieve the most accurate testing results on any instrument, on any sample, at any time.

Reference system and centralised calibration for milk (payment) testing

Dave Barbano
Cornell University
Ithaca, NY

Outline

- PreCalibration
- Homogenizer Performance Evaluation
- Calibration Samples
- Research to Improve Accuracy of Infrared Milk Analysis

PreCalibration (monthly)

Key Parameters

- Flow system check
- Homogenization efficiency
  - evaluated by particle size analysis
- Water and milk repeatability
- Primary slope for each component
- Purging efficiency
- Linearity
  - (evaluated with modified milk samples)
- Intercorrection values
  - (evaluated with modified milk samples)

Outline

- PreCalibration
- Homogenizer Performance Evaluation

Homogenization Efficiency Testing (monthly)

Three vials pasteurized, unhomogenized milk are sent from Cornell to each lab per instrument each month.

The milk is warmed to 42°C, pumped through the instrument and the instrument homogenized is collected from the by-pass outlet, immediately cooled, and shipped back to Cornell. Each samples is test by laser light scattering to determine the fat globule size distribution. We recommend that a lab replace the homogenizer when the the d(0.9) of the particle size distribution reaches 1.7 microns.

Homogenization Efficiency Testing (monthly)

Recently, we have also started investigating why homogenizers fail. Laboratories send the failed homogenizer to Cornell and we disassemble the homogenizer. We conduct a microscopic examination of the internal parts to try to determine the cause of the homogenizer failure.

Also, when possible, we check the performance of new homogenizers before they are installed on an instrument.

Primary Slope Control (monthly)

When primary slope (i.e., gain) of the primary signal for each measured component is set in a one to one relationship with the change in concentration of that component, the intecorrection factors from one instrument the next become almost identical, particularly among FTIR instruments run in traditional filter mode.

Outline

- PreCalibration
- Homogenizer Performance Evaluation
- Calibration Samples
  - Production of modified milk samples
  - All lab mean chemistry reference values
  - Chemistry method proficiency testing and trouble shooting.
  - Stability of instrument performance and slope intercept values.

Production of Modifed Milk Calibration Samples

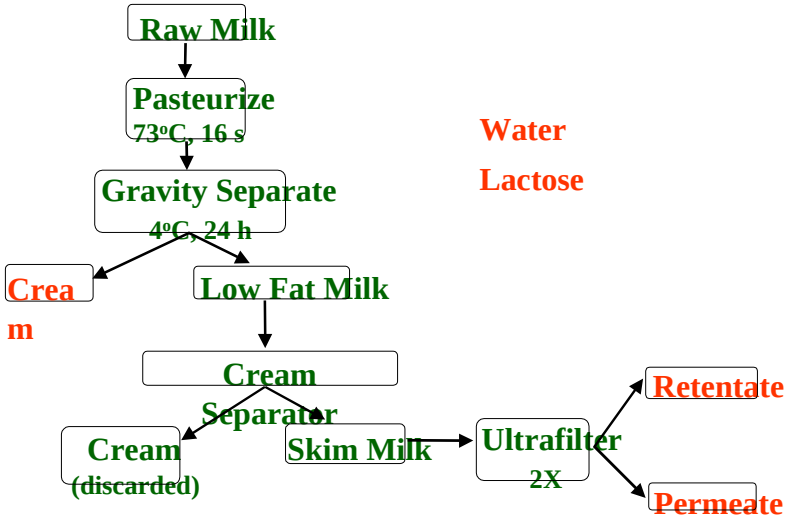
14 milks - an orthogonal matrix of composition with no correlation among component concentrations.

- Fat range = 0.2 to 5.8%
- True protein range = 2 to 4.3%
- Anhydrous lactose range = 3.9 to 5.2%

These samples are used to set slope and intercept of the intercorrected mid-IR signal.

One calibration can be used for raw milk payment testing and for testing of homogenized HTST pasteurized milks (0.2 to 3.6% fat).

Production of Ingredients for Milk Calibration Samples



Formulation of Calibration Samples

The composition of each ingredient is entered into an Excel spreadsheet.

The target composition of each of the 14 samples is pre-set in the spreadsheet.

The optimization solver function of Excel is used to calculate the amount of each ingredient needed for each of the 14 samples to achieve the compositions targets.

Currently, originally the samples were preserved with potassium dichromate, currently the samples are preserved with Microtabs II (bronopol and delvocid) and have a refrigerated shelf-life of 1 month.

Example: Modified Milk Calibration Sample Set – June 2, 2008

Sample	Fat	Protein	Solids	Lactose
1	0.2115	4.2463	9.5861	4.0373
2	0.6432	2.2219	8.4074	4.5166
3	1.1157	3.9037	11.3312	5.1119
4	1.5164	2.5634	10.1098	4.9405
5	1.9464	3.5745	10.9290	4.3015
6	2.3774	2.9012	10.9171	4.5483
7	2.8082	3.2422	11.7286	4.5522
8	3.2425	3.0787	11.8243	4.4113
9	3.6722	3.4097	12.9167	4.6744
10	4.1084	2.7470	11.9975	4.1303
11	4.5460	3.7498	14.3034	4.8308
12	4.9743	2.4132	12.3422	3.9908
13	5.4067	4.1000	15.2816	4.5721
14	5.8312	2.0783	14.0166	5.0522
Mean	**3.0286**	**3.1593**	**11.8351**	**4.5479**
min	**0.2115**	**2.0783**	**8.4074**	**3.9908**
max	**5.8312**	**4.2463**	**15.2816**	**5.1119**
range	**5.6197**	**2.1681**	**6.8742**	**1.1211**

14 Modified Milk Samples (Three Purposes)

First, each month the testing of these samples provides a proficiency test of the fat by ether extraction, the true protein by Kjeldahl, the anhydrous lactose by enzymatic, and total solids by oven drying methods. The orthogonal matrix of composition of the set of samples provides some interesting diagnostic and trouble shooting opportunities that are used to improve the performance of the laboratories that run the chemistry methods.

The performance of individual laboratories and the group of laboratories for the chemistry methods has been improved.

14 Modified Milk Samples (Three Purposes)

Second, the all-laboratory mean with outliers removed is used to create a fat, protein, and lactose reference value for each sample.

Third, the samples are used for 1 month to set the slope and intercepts for each instrument. Because of the orthogonal matrix of composition, the data can be used to evaluate and adjust the linearity and intercorrection response of each instrument. These evaluation calculations and protocols are built into a software package we have written called IR-QC.

14 Modified Milk Samples

Instrument Calibration Performance:

Standard Deviation of the Difference (SDD) between Reference Chemistry and Instrument Predictions

Before we started using the modified milks, the SDD with producer calibration samples generally were never less than 0.025% for any component.

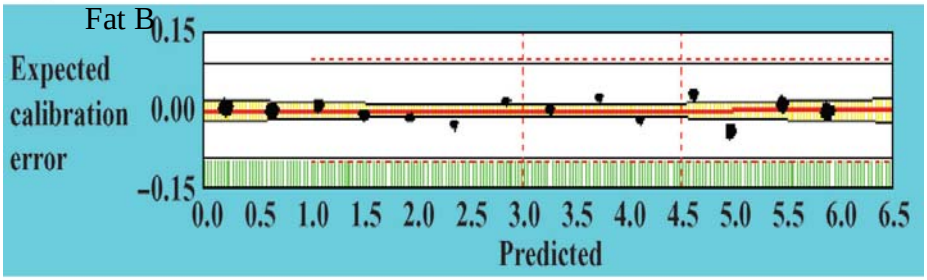
14 Modified Milk Samples

Instrument Calibration Performance:

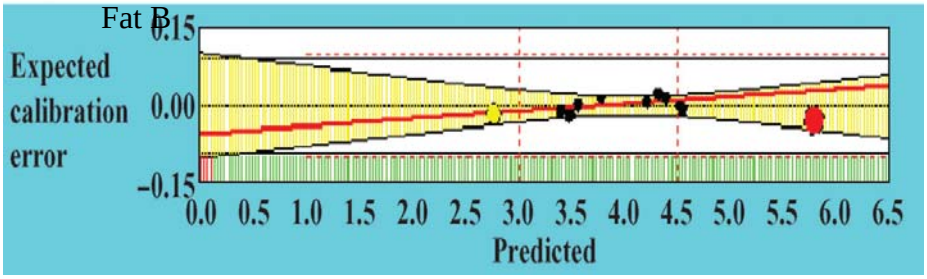
With Modified Milks and all-lab mean reference values, the SDD on all components is < 0.015% and often < 0.01%.

The size of the 95% confidence interval around the slope of the regression line has been reduced greatly by the use of the modified milk calibration samples.

a) Modified Milk Calibration – Fat B



b) Producer Milk Calibration – Fat B



Outline

- PreCalibration
- Homogenizer Performance Evaluation
- Calibration Samples
- Research to Improve Accuracy of Infrared Milk Analysis

Research to Improve Accuracy of Infrared Milk Analysis

- Development of an optimized set of traditional “virtual” sample and reference filter wavelengths for use in FTIR instruments. – status: complete and in process of publication.

Research to Improve Accuracy of Infrared Milk Analysis

- Quantitative determination of the impact of variation in fatty chain length and unsaturation on Fat B and Fat A on absorbance at sample and reference wavelengths with a model sample system. – status: complete and in the process of publication.
- Verification of the chain length and unsaturation impacts with producer samples. – status: complete and in the process of publication.

Research to Improve Accuracy of Infrared Milk Analysis

- Develop an improved traditional “virtual filter” calibration approach that minimizes the impact of variation in fatty acid chain length and unsaturation. - status: work in progress.

Research to Improve Accuracy of Infrared Milk Analysis

- Determine the impact of various preservatives on infrared uncorrected signals initially and during calibration sample shelf-life – status: data collection is complete.
- Develop a set of unpreserved modified milk samples that have a refrigerated shelf life of 1 month – status work in progress with some success.

Research to Improve Accuracy of Infrared Milk Analysis

- Continue to implement and apply new statistical quality control tools in IR-QC to calibration data to improve the accuracy of milk testing.

Acknowledgments

- Test Procedures Committee of the USDA Federal Milk Markets.
- Laboratory staff at Cornell and the USDA Federal Milk Market laboratories and affiliated laboratories.
- Mid-infrared equipment manufacturers for their support and collaboration.

Assessment of Lab Performance and Analytical Equivalence in Milk Testing in North America

Paul Sauvé

Canadian Lab Services, Ottawa, Canada

Abstract

Statistics on milk recording laboratories of North America and analytical methods under control are presented so as to introduce and compare respective laboratory certification/accreditation systems and laboratory performance evaluation in Canada and US and Mexico. Respective systems are implemented and monitored by closely coordinated organisations, Canadian Laboratory Services for Canada and Quality Certification Services for United States and Mexico. The principles and organisations as well as proficiency testing schemes appear very close assuring consistency between North American countries.

Assessment of Lab Performance and Analytical Equivalence in Milk Testing in North America

ICAR, June 16, 2008

Niagara Falls, New York

Paul Sauvé

Canadian Lab Services

Capital Laboratory Services

Ottawa, Ontario, CA

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DHI Testing in North America - Facts and Figures

- 55 official (accredited/certified) DHI laboratories in North America
- 44 in USA (including 1 in Puerto Rico), 8 in Canada, 1 in Mexico *
- approximately 115 infrared analyzers (fat, protein, MUN)
- approximately 130 somatic cell counters
- approximately 40 MUN analyzers (IR, differential pH, FIA, etc.)
- approximately 70,000,000 samples tested annually *
- several DHI labs offering additional services

(forage analysis, Johnes screening, water analysis, component payment testing, drug residues, bacteria, added water, vet services, nutritional consulting, etc.)

Accreditation / Certification

Canada

- DHI labs are accredited under ISO 17025.
- Accreditation is delivered by the *Standards Council of Canada* and coordinated by *CLS.*
- On-site assessments are conducted every two years.

USA and Mexico

- DHI labs are certified under guidelines developed by the *Council on Dairy Cattle Breeding.*
- Certification is delivered and coordinated by *QCS.*
- On-site audits are conducted every two years.

Proficiency Testing Programs

Canada

•Analytical performance is assessed monthly using samples provided by *Canadian Lab Services*.

• Data analysis and reporting is coordinated by *CLS*.

USA and Mexico

• Analytical performance is assessed monthly using samples provided by *Eastern Lab Services*.

• Data analysis and reporting is coordinated by *QCS* with involvement of an outside contractor.

Proficiency Testing - Schedules and Samples

Canada

• Six times annually sets of 20 blind duplicate samples are circulated according to a pre-arranged schedule.

• Six times annually sets of 16 individual samples are circulated unannounced. *

• Samples are sent by overnight courier.

USA and Mexico

• Every month sets of 24 duplicate samples are circulated according to a pre-arranged schedule.

• Samples are sent by overnight courier.

Proficiency Testing - Data submission and Reporting

Canada

• Test results are submitted electronically and performance reports are returned electronically.

• Reports include coded data from all participating labs.

• Turn around time from deadline to circulation of reports < 3 days.

USA and Mexico

• Test results are submitted and reports are returned via a secure web site.

• Reports include individual data and summary graphs from all labs.

• Turn around time < 3 days.

Proficiency Testing - Components included

Canada

• 6 times annually: fat, protein, lactose, total solids, MUN, SCC

• 6 times annually: fat, protein, SCC, MUN

• monthly: MUN

• A program for Johnes screening is under development.

USA and Mexico

• monthly: fat, protein, SCC, MUN *

• A program for Johnes screening is under development.

Proficiency Testing - Tolerances (fat and protein)

Canada

- MD < +/- .04% and SDD < .04% in three of the last four trials
- RMD (rolling mean difference) < .02% across the last six trials

USA and Mexico

- MD < +/- .04% and SDD < .04% in three of the last four trials
- RMD (rolling mean difference) < .02% across the last six trials

Proficiency Testing - Tolerances (SCC)

Canada

- M%D < +/- 10% and SD%D < 10% in three of the last four trials
- RM%D (rolling mean difference) < 5% across the last six trials

USA and Mexico

- M%D < +/- 10% and SD%D < 10% in three of the last four trials
- RM%D (rolling mean difference) < 5% across the last six trials

Proficiency testing - Example

Sample	Fat (ref)	Fat (IR)	Diff.
1	3.685	3.715	0.030
2	3.790	3.820	0.030
3	3.882	3.910	0.028
4	3.898	3.910	0.012
5	3.998	4.035	0.037
6	4.006	4.040	0.034
7	4.063	4.105	0.042
8	4.157	4.170	0.013
9	4.286	4.300	0.014
10	4.368	4.395	0.027
		MD	0.027
		SDD	.0010

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Proficiency testing - Example

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		MD	0.027
		SDD	.0010

MD < +/-.04% in three of the last four trials

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Proficiency testing - Example

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9	4.286	4.300	0.014
10	4.368	4.395	0.027
		MD	0.027
		SDD	.0010

SDD < .04% in three of the last four trials

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Proficiency Testing - Example

• The rolling mean difference (RMD) must be less than .02 percent across the last six trials.

Date	MD
Jan 2008	-0.020
Feb 2008	0.015
Mar 2008	0.022
Apr 2008	-0.031
May 2008	0.024
Jun 2008	0.011
RMD	0.004

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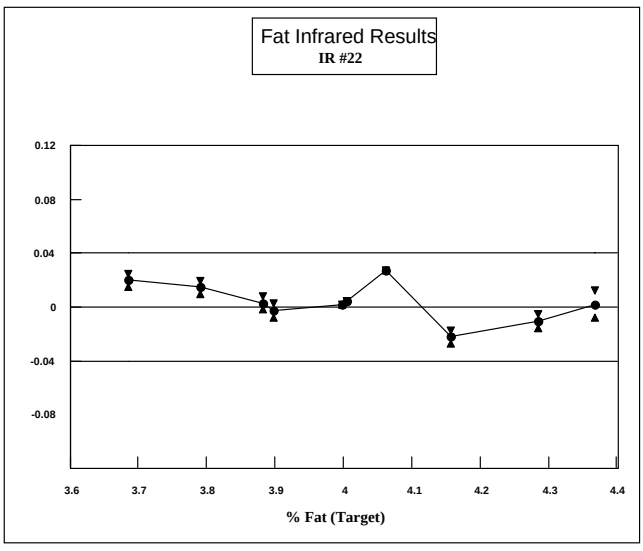
Canadian Program - Individual Data, Tabular Presentation

Fat Infrared Results **IR #22** **May 2008**

SAMPLE	TARGET	REP. 1	REP. 2	MEAN	RANGE	SD	RES.
MS-2	3.685	3.710	3.700	3.705	0.010	0.007	0.020
MS-8	3.790	3.810	3.800	3.805	0.010	0.007	0.015
MS-6	3.882	3.880	3.890	3.885	0.010	0.007	0.003
MS-9	3.898	3.890	3.900	3.895	0.010	0.007	-0.003
MS-7	3.998	4.000	4.000	4.000	0.000	0.000	0.002
MS-10	4.006	4.010	4.010	4.010	0.000	0.000	0.004
MS-4	4.063	4.090	4.090	4.090	0.000	0.000	0.027
MS-5	4.157	4.130	4.140	4.135	0.010	0.007	-0.022
MS-3	4.286	4.270	4.280	4.275	0.010	0.007	-0.010
MS-1	4.368	4.380	4.360	4.370	0.020	0.014	0.002
						MD	0.004
						SDD	0.014
						SDA	0.007

Canadian Program - Individual Data, Graphical Presentation



Canadian Program - Summary Table

Protein Infrared Results

IR #	MD	SDD	SDA	S
IR #2	-0.036	0.013	0.009	0.023
IR #14	-0.021	0.031	0.006	0.022
IR #6	-0.017	0.026	0.009	0.019
IR #13	-0.010	0.026	0.005	0.016
IR #19	-0.009	0.032	0.015	0.021
IR #10	-0.005	0.025	0.006	0.015
IR #3	-0.004	0.026	0.009	0.016
IR #17	-0.003	0.029	0.003	0.017
IR #8	-0.003	0.026	0.006	0.016
IR #11	-0.002	0.027	0.007	0.016
IR #22	-0.002	0.025	0.007	0.015
IR #12	0.000	0.026	0.007	0.016
IR #18	0.001	0.027	0.004	0.016
IR #4	0.001	0.027	0.005	0.016
IR #5	0.001	0.025	0.004	0.015
IR #1	0.001	0.012	0.005	0.008
IR #15	0.005	0.024	0.008	0.015
IR #20	0.005	0.017	0.005	0.011
IR #7	0.009	0.031	0.003	0.019
IR #21	0.009	0.012	0.002	0.009
IR #16	0.014	0.026	0.007	0.018
IR #9	0.021	0.030	0.007	0.022
			Mean	0.016

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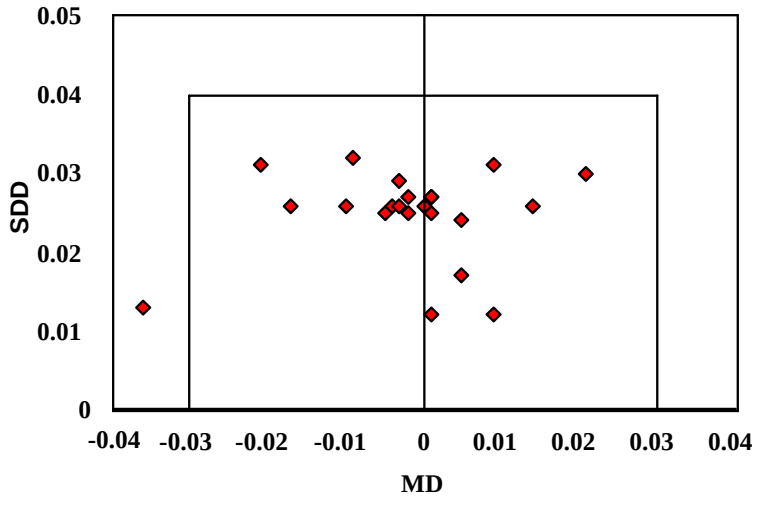
Canadian Program - Summary Graph



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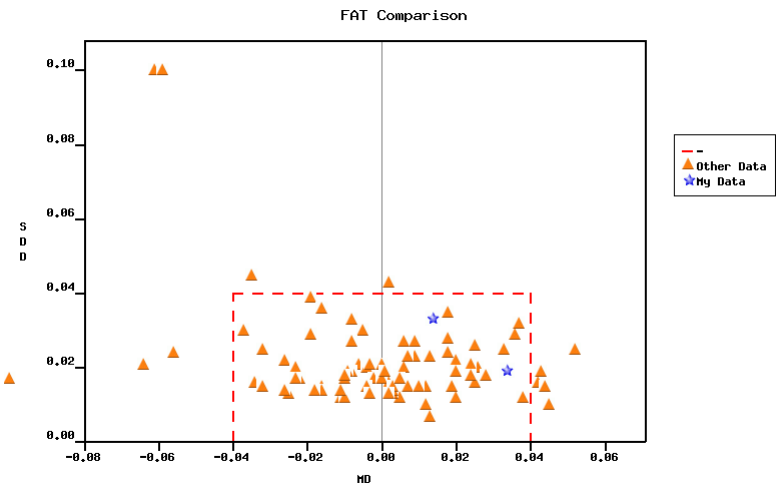
QCS Program, Individual Data, Tabular Presentation

B2500 A
Butterfat

Sample Number	Lab/Instrument Ref	Inst	Avg Diff	Instr Results Rep1	Rep2	Prec Stats Range	SD	Reps	Accuracy Stats IR Mean	Diff
1	3.557	3.564	0.007	3.56	3.58	0.020	0.014		3.570	0.013
2	3.907	3.909	0.002	3.91	3.90	0.010	0.007		3.905	-0.002
3	2.990	2.990	0.000	3.01	3.03	0.020	0.014		3.020	0.030
4	4.153	4.138	-0.015	4.20	4.20	0.000	0.000		4.200	0.047
5	3.547	3.550	0.003	3.57	3.59	0.020	0.014		3.580	0.033
6	3.797	3.782	-0.015	3.78	3.79	0.010	0.007		3.785	-0.012
7	3.707	3.723	0.016	3.74	3.77	0.030	0.021		3.755	0.048
8	3.223	3.224	0.001	3.26	3.26	0.000	0.000		3.260	0.037
9	3.640	3.649	0.009	3.68	3.67	0.010	0.007		3.675	0.035
10	4.297	4.289	-0.008	4.28	4.27	0.010	0.007		4.275	-0.022
11	4.817	4.800	-0.017	4.75	4.76	0.010	0.007		4.755	-0.062
12	4.370	4.382	0.012	4.40	4.39	0.010	0.007		4.395	0.025
		MD	0.000				SDA	0.006	MD	0.014
		SDD	0.011						SDD	0.033

QCS Program - Summary Graph



QCS Program - Historical Data, Tabular Presentation

	FAT Results		
Month	MD	SDD	RMD
Jun	-0.011	0.020	0.002
Jul	0.009	0.015	0.008
Aug	-0.027	0.023	0.004
Sep	-0.004	0.011	0.002
Oct	0.005	0.018	-0.002
Nov	0.006	0.023	-0.004
Dec	0.022	0.018	0.002
Jan	-0.015	0.022	-0.002
Feb	-0.037	0.020	-0.004
Mar	0.006	0.020	-0.002
Apr	-0.025	0.018	-0.007
May	0.014	0.033	-0.006

Changed cell here!

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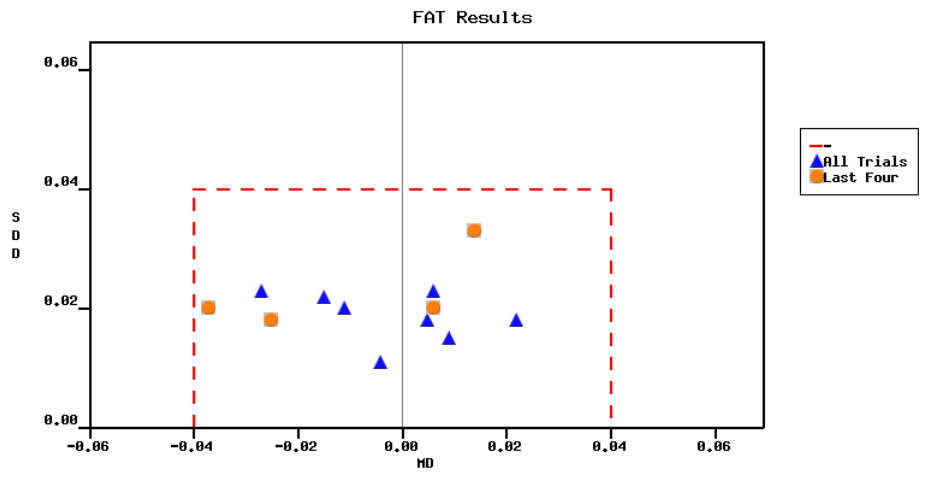
QCS Program - Historical Data, Graphical Presentation



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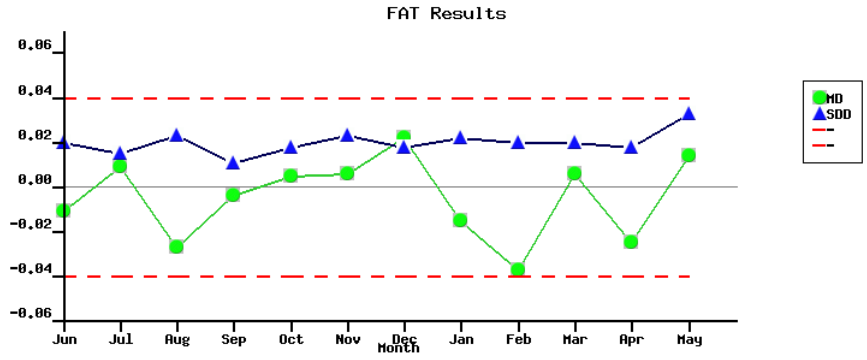
QCS Program - Historical Data, Graphical Presentation



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Discussion and conclusion

Discussion was focused on the centralised calibration issue.
Matrix effect with mid infrared analysis in relation with milk composition as depending on feeding practices and available forages in the collect area was found relative. Centralised calibration made for large areas (region, countries) and marked geographical differences are likely to show more variation in animal foodstuff and quality.

Centralised calibration for a large area suits better with only little local effect otherwise, if applyed with no correction of region biases, the level of uncertainty is larger but can accepted for milk recording testing results at a certain degree provided it is prior evaluated.

A question was on how far multivariate calibration applyied to the whole MIR spectrum absorbances with using milk samples of various region could overcome the regional effects. There is no recent information on that nevertheless, it was agreed on that if new instruments have drastically improved accuracy through optimised fittings and reducing marginal interferences, main fundaments of MIR analysis for major components of milk keeps the same with still matrix effects sensitivity. Also such new devices are not generalised and many classical filter instruments are still used and this for a while before complete replacement. Moreover newly appearing feeding stuff with special nutrient to favour unsaturated fatty acid in milk fat can produce even more discrepancy within and beween collect areas.

To the question on the efficiency of sample sets, it is explained multivariate calibration using natural milk samples normally serve to calculate internal coefficients made to reduce accuracy standard deviation but they are not so adequate as recombined (or modified) milk samples to adjust accurately the calibration line as the speakers' presentations showed.

The point of the difficulty in identifying proper representative samples for calibration was raised. Answer was that a commingling of bulk milk of the area was appropriate provided physicochemical quality is assured before the testing operation.

Chairman concluded by considering with satisfaction the presentations of the second part of the meeting had shown a large consensus on the technical tools and methods presented in the first part with a number of them already adopted and used from years. This fact justifies to produce guidelines to stick on the paper optimum procedures.

Conclusion of the meeting

The meeting was the occasion to make a review of of the today situation of ICAR Reference Laboratory Network so as it can be better known in North America and favour collaboration with North American laboratory networks. The goal seemed reached and commitment taken to kave further meetings with NALMA.

It was also the occasion to explain the principle of the international traceability of reference results and the anchorage of routine laboratories via national reference laboratories based on the concrete reality of proficiency studies. Thanks to the information it is expected more numerous ICAR countries to nominate reference laboratories and involve them in ICAR international proficiency studies. Reference system and centralised calibration have been presented as practical, easy and economic tools for a national laboratory network, and also promising in the field of forthcoming on-farm analysis.

Presentations will serve to define appropriate ICAR guidances on proficiency study organisation and centralised calibration.

The Chairman thanked the speakers and the attendance for their large participation and invited every attending person to take part in the joint meeting of NALMA / ICAR Reference Laboratory Network in the afternoon.