



**Third ICAR
Reference Laboratory Network Meeting**

**Kuopio - Finland
6 June 2006**

MTL WG

***ICAR Working Group on
Milk Testing Laboratories***

FOREWORD

ICAR Reference Laboratory Network is now in existence for ten 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, and this Year 2006, in June, at the 35th ICAR Session in Kuopio-Finland with the programme presented in the following pages.

Year 2006 appears as a milestone in the implementation/development of the AQA system of ICAR. Ten years have passed from the launching of the laboratory network and twelve from the start-up of the implementation programme, so was it felt of great interest to measure progress made, evaluate the present stage and prepare the future through some prospects.

Nowadays ICAR is implementing a world wide system for recognition of quality in various technical areas of its field of activities in which milk recording analysis and dairy laboratories aspects take part. To meet ICAR expectation - i.e. to make national situation comparable thus reach equivalence between countries -, laboratory harmonisation has to carry on and be strengthened within and between countries. Besides, the analytical landscape evolves with the next advent of analytical devices/systems in farms and needs are for alternative ways of thinking about milk analysis, its location, precision needed, harmonisation, consistency with today's situation ... These considerations have served as bases to define the programme of ICAR Reference Laboratory Network Meeting in 2006.

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, 28th June 2006

Olivier Leray
Chairman of ICAR Working Group
on Milk Testing Laboratories

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Role and objectives of the network and evolution from 1996

Olivier Leray

Cecalait, Poligny (FR)

The ICAR Reference Laboratory Network was created in 1996 to become the basis of an international analytical quality assurance system for milk recording. It was aimed to serve as a communication tool and a vector of services that can help in harmonising analytical practices and milk recording results in ICAR member countries.

It is a continuous task nevertheless begun through the definition by ICAR of relevant standards and recommendations worldwide accepted - can they be IDF/ISO standards or ICAR Guidelines - and their distribution to ICAR countries through their national reference laboratories that then can put them in application. It is also a technical medium to provide analytical traceability to routine laboratories – meaning establishing a link with trustworthy true values (so-called reference values) - and to either improve or at least maintain laboratory performances within tight acceptable ranges of error.

It is based on a laboratory network structuring on two (possibly three) levels, international, national (and possibly regional). At the first level operates an international reference laboratory network made of pilot or master laboratories of every country that have technical/scientific experience and knowledge so as to monitor routine laboratories and supply routine with various technical services or tools for analytical quality assurance (QA) or quality control (QC) in their countries. The second level is made of the routine laboratories that constitute national (routine) laboratory networks, generally one per country. A third may be foreseen in special cases of federal countries where regions where every region can organise a network if needed or in the perspective of future in-farm analytical devices and their possible organisation in sub-network monitored by the central laboratories of the sector or region.

Analytical harmonisation missions are prerequisites to join ICAR Reference Laboratory Network with special exception made for those countries which have no more than two routine laboratories and therefore can be connected directly to the international level. Through the networking principle the intent is also to enable collaboration and experience sharing for the benefit of AQA system development in respective countries.

The numbers of laboratories qualified for various scientific/technical mission have increased gradually till 2003, during the three last years, and keep stable since. Nevertheless, the numbers of competence continue to grow meaning the network development is going on with respect to the qualification of members.

In mid 2006, there are 37 members of 31 countries all involved in cow milk analysis, among which 13 laboratories work also for sheep milk and 14 also for goat milk.

MTL WG

*ICAR Working Group on
Milk Testing Laboratories*



ICAR Reference Laboratory Network

- 3rd Meeting, Kuopio, 6 June 2006

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- Agenda -

- 8.30 : Opening - Welcome - Round table for presentation
Introduction : Composition and evolution of the network since 1996 (O. Leray)
- 8.50 : ICAR AQA strategy and prospect for the network (O. Leray)
- 9.10 : Information on quality assurance policy of ICAR and view on milk analysis aspects (A. Rosati)
- 9.30 : Discussion
- 10.00 : Break (coffee, tea, drinks)
- 10.20 : Reference system - Principle and practice (C. Baumgartner)
- 10.40 : Reference and calibration systems for routine milk testing - Advantages/Disadvantages, choice (O. Leray)
- 11.00 : Example of national reference system and centralised calibration (J. High)
- 11.20 : What is the required Accuracy of a Test related to Genetic Improvement (H. Wilmink)
- 11.50 : Discussion - Conclusion of the meeting
- 12.20 : Closure

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- INTRODUCTION - GENERAL OBJECTIVES -

- **History :** From 1994, a new ICAR policy for AQA
 - Develop an international AQA system for DHI within ICAR based on harmonised laboratory practices.
 - Provide confidence and allow between country comparison and international genetic index calculation with regards to analytical data.
- **Implementation by MTL WG :**
 - Harmonisation of analytical practices :
 - » Analytical methods
 - » Analytical Quality Assurance
 - » Analytical performances and traceability of precision

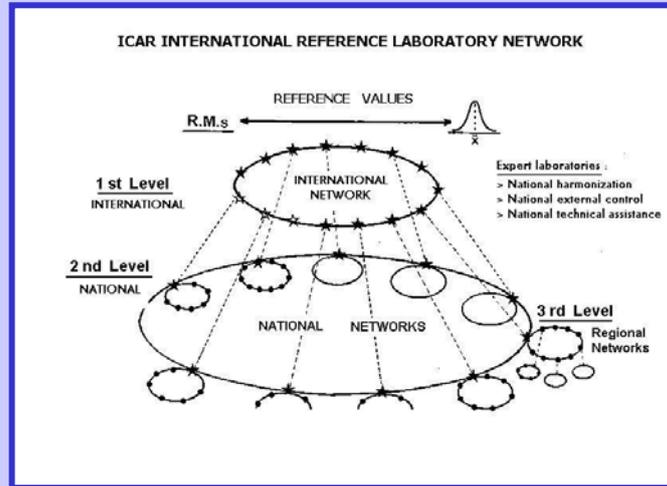
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ROLES OF THE NETWORK

- ICAR Reference Laboratory Network is expected to operate as :
 - an **international platform** for diffusing GLP and AQA based on international guides and standards => **communication**
 - the instrument for defining **international consensual so-called « true values »** to refer to and provide the **precision traceability** to routine labs via network members => **International Proficiency Studies**
 - a mean for developing collaborations for laboratory purposes => **Co-operation.**

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THEORETICAL STRUCTURE



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Missions / activities expected - Eligibility criteria -

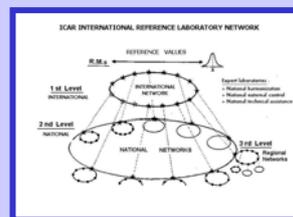
- 1- National ring test organizer
- 2- Reference Material supplier
- 3- Master laboratory for centralized calibration
- 4- Teaching and training in laboratory techniques
- 5- Information on analytical methods
- 6- Evaluation of analytical methods/instruments
- 7- Research on analytical methods
- 8- National 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 2006



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ICAR Reference Laboratory Network

Membership

37 laboratory members from **31** countries :

Argentina (1)	Austria (1)	Belgium (2)	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 (1)
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 (2)	U.S.A. (2)	Zimbabwe (1)	

(n) : number of member(s)

among which :

- 37 members for cow
- 14 members for goat
- 13 members for sheep

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ICAR Reference Laboratory Network - Evolution since 1998 -

Evolution of the composition and national roles from 1998 to 2006

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

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

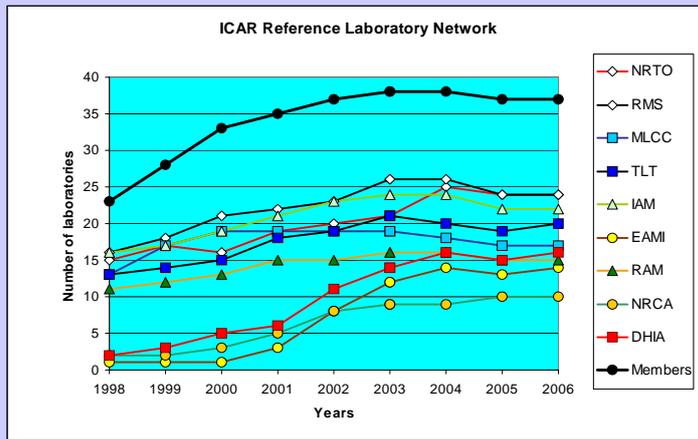
MLCC = Master Laboratory for Centra
 EAM = Evaluation of Analytical Method
 DHIA = Dairy Herd Improvement Analy
 Payment = Analyses for milk payment

Evolution of the proportions of national roles from 1998 to 2006

YEAR	NRTO	RMS	MLCC	TLT	IAM	EAMI	RAM	NRCA	DHIA	PAYMENT	Other anal.
1998	68	73	59	59	73	5	50	9	9	5	5
1999	63	67	63	52	63	4	44	7	11	4	4
2000	48	64	58	45	58	3	39	9	15	3	3
2001	54	63	54	51	60	9	43	14	17	6	3
2002	54	62	51	51	62	22	41	22	30	14	3
2003	55	68	50	55	63	32	42	24	37	18	8
2004	66	68	47	53	63	37	42	24	42	24	8
2005	65	65	46	51	59	35	41	27	41	22	8
2006	65	65	46	54	59	38	41	27	43	27	8

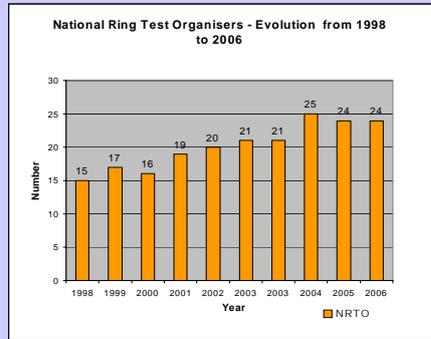
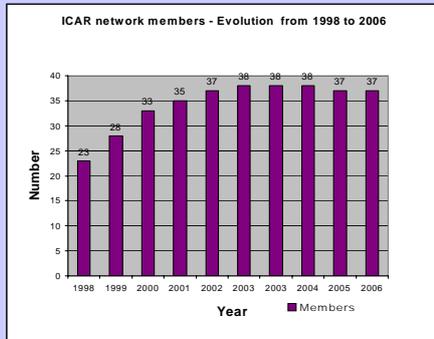
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Evolution of membership and missions/activities from 1998 to 2006



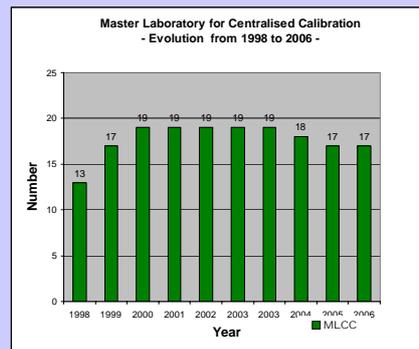
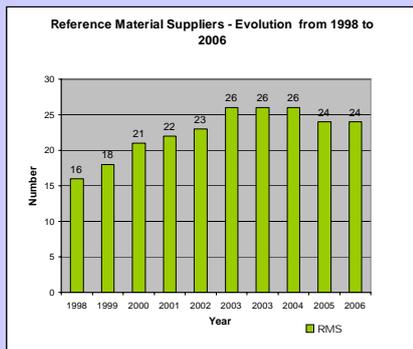
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Evolution of membership and missions/activities from 1998 to 2006



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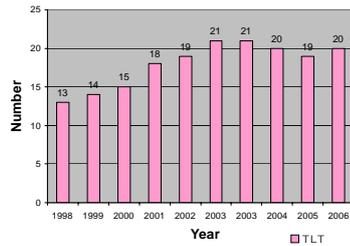
Evolution of membership and missions/activities from 1998 to 2006



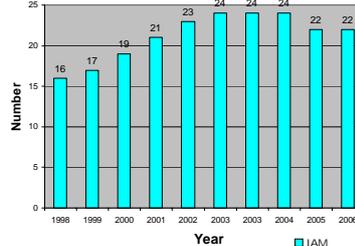
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Evolution of membership and missions/activities from 1998 to 2006

Training in Laboratory Techniques
- Evolution from 1998 to 2006 -



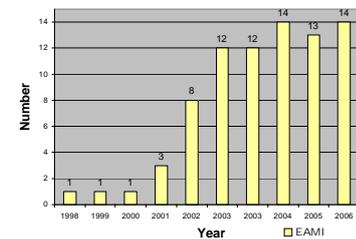
Information on Analytical Methods
- Evolution from 1998 to 2006 -



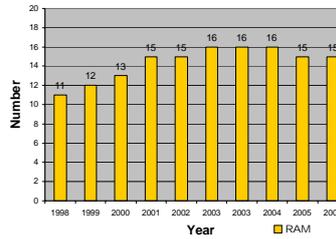
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Evolution of membership and missions/activities from 1998 to 2006

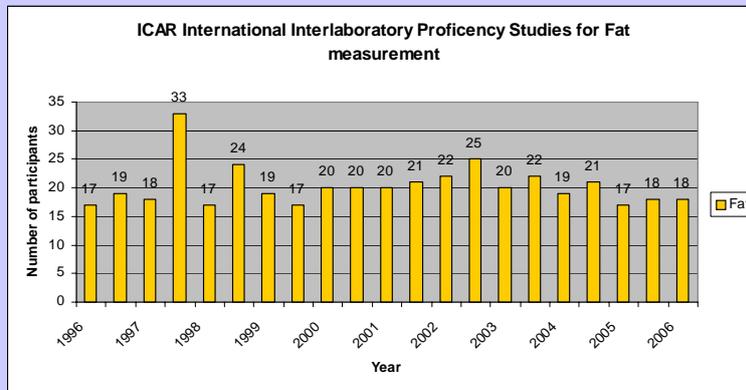
Evaluation of Analytical Methods & Instruments
- Evolution from 1998 to 2006 -



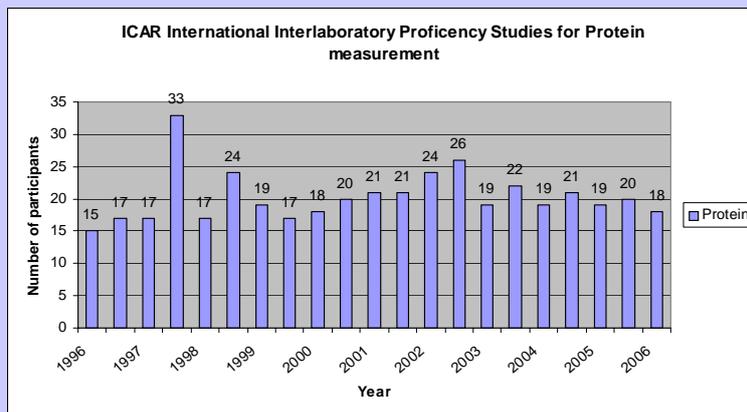
Research in Analytical Methods
- Evolution from 1998 to 2006 -



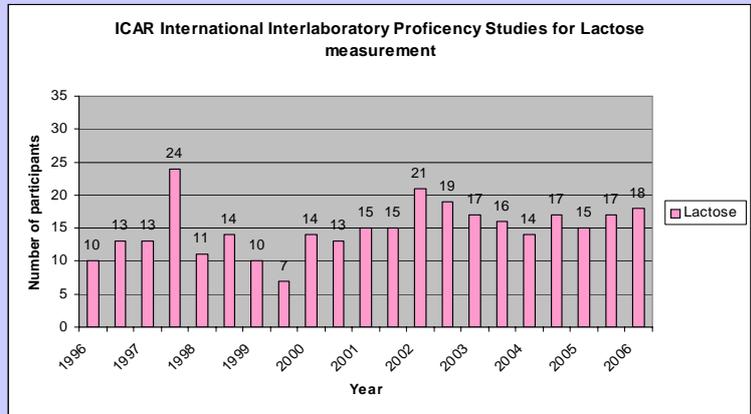
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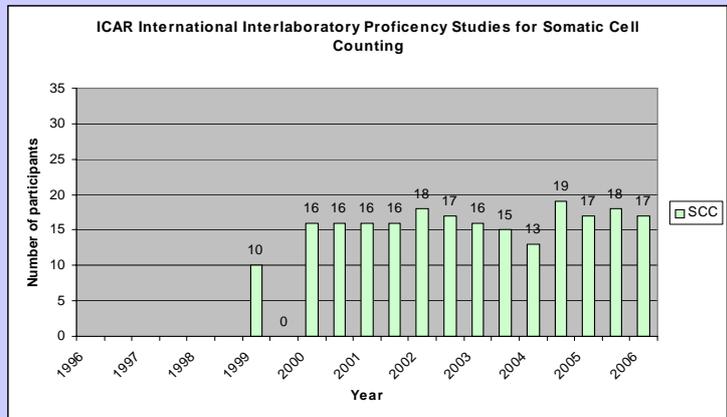
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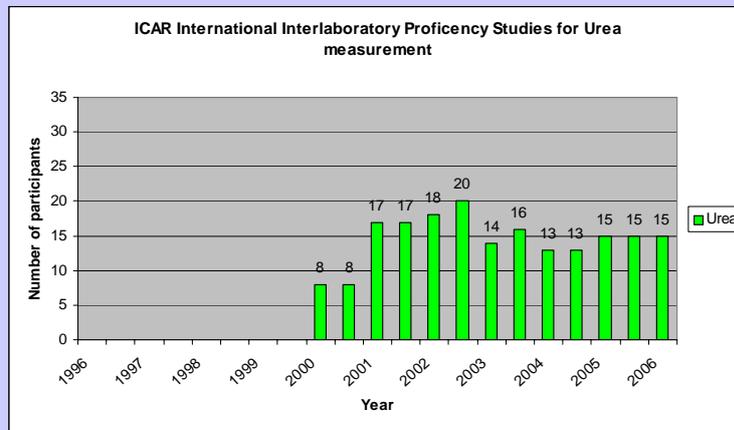
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CONCLUSION ON THE NETWORK IMPLEMENTATION

Nominations by national organisations :

have reached a plateau

⇒ indicates the phase of implementation and growth completed

International Proficiency Testing schemes :

followed at a regular rate by the members of the laboratory network

⇒ confirms the interest in harmonisation and congruent analytical performances between countries

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ICAR AQA strategy and prospective for the network

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Introduction

Year 2006 marks the 10th anniversary of ICAR Reference Laboratory Network and is also 12 years past from the decision of starting-up an international AQA system for milk recording in ICAR Session in Ottawa (1994).

The Programme drawn in Ottawa was to design and implement a international framework for quality assurance in milk recording analysis and harmonisation of practices to ensure equivalence of results worldwide. Implemented by the Working Group on Milk Testing Laboratories of ICAR (MTL WG), it contained the following items:

- 1- Definition of reference methods by reference to ISO/IDF international standards (ICAR guidelines)
- 2- Definition of routine methods by reference to a validation process (ICAR approval protocol)
- 3- Definition of quality assurance to apply to methods : External & internal monitoring for both reference & routine methods (ICAR guidelines)
- 4- Promoting a frame and model for routine lab monitoring by national reference laboratories federated in an international network .
- 5- Harmonising countries with regard to reproducibility of reference methods used for calibration by international proficiency testing.

The frame designed is made of minimum two levels of operation:

- national network of routine laboratories : reference laboratories work to harmonise and monitor routine labs in their countries.

- international network of reference laboratories : the network serves to harmonise reference labs as a collective tool with intended roles for providing some traceability of so-called “true” values determined by the international level through collaborative studies (lab performance evaluation).

The model proposed is based on a list of competence or missions – so-called eligibility criteria – possibly assigned by ICAR national organisations to their nominated laboratories :

- | | | | |
|---|--------|---|--------|
| 1- National ring test organizer | (NRTO) | 5- Information on analytical methods | (IAM) |
| 2- Reference Material supplier | (RMS) | 6- Evaluation of analytical methods/instruments | (EAMI) |
| 3- Master laboratory for centralized calibration | (MLCC) | 7- Research on analytical methods | (RAM) |
| 4- Teaching and training in laboratory techniques (TLT) | | 8- National regulatory control of DHI analyses | (NRCA) |

Conclusion of the first period (1994-2006) - Implementation of the ICAR AQA system

Involvement in the proposed system is made on a voluntary basis of each ICAR country with yearly invitation to participate in the network. ICAR through MTL WG made the demonstration on its capability to provide a regular collective service to its members which was concluded by an evaluation of precision findings over period the 1996-2003.

From 2003, the network membership has not increased any more. Nevertheless, new competence appearing has been noted which indicates the development process is continuing nevertheless more oriented to the qualification of members along time by additional technical value.

Meanwhile technical documents as guidelines and protocol were produced :

- Guidelines for quality assurance in milk analysis, 1998, updated and published in ICAR guidelines in 2006,

- List of methods for milk recording analysis (2002, 2006)
- Milk analyser evaluation protocol (2002), the relevant approval procedure and implementation of approval process (2006)

Technical tools were developed and presented for possible use by ICAR laboratories but also for further collective system development:

- recombined calibration milk samples for fat, protein, lactose determination by infra red,
- recombined calibration milk samples for somatic cell counting by fluoro-opto-electronic methods
- deep-frozen calibration milk samples for long preservation.

Prospect for the second period (2006->) - Enhancement of the ICAR AQA system

The first period has shown the involvement of a significant part of dairy countries of ICAR through the nomination of 37 reference laboratories of 31 countries. From them up to 23 reference laboratories have been regularly involved in ICAR proficiency trials.

Nowadays, the AQA frame being completed, it is felt opportune to strengthen the AQA system by giving clear models to countries for national use and strengthening the role of international network :

1- Strengthening the international network role :

Quality assurance is an issue for ICAR and milk analysis is part of its activities which would result in the duty of ICAR member countries to :

- ⇒ involve with the laboratory network through member laboratory nomination.
- ⇒ commission their national reference laboratories to participate systematically in the proficiency testing scheme organised by ICAR.

This can be effective by becoming an element of a quality recognition by ICAR, for instance the ICAR Special Stamp granting or, from 2006, entering the Quality Certification system newly implemented by ICAR.

2- Enhancing harmonisation Standard QC/QA tools for countries :

- ⇒ Standardised procedures (guides) for proficiency studies : Comparable efficiency between national schemes relates to similar procedures to apply interlaboratory proficiency testing. Nowadays international guides and standard provide general recommendations that need to be adapted in order to limit discrepancy between national schemes. Moreover it will work for better equivalence in accreditation between countries in this sector.
- ⇒ Standardised procedures (guides) for centralised calibration systems: Accuracy of calibration is determined by various aspects mainly related to calibration sample. Elements for choice of a calibration system can be provided as well can be defined optimal preparation procedure for calibration samples. Centralised calibration can be an optimal situation in many situation for milk recording. Also the advent of milk analysis in the farm will require to develop practical tools to check and adjust calibration of analytical devices. Centralised calibration associated to centralised reference system and monitoring will constitute the only way for harmonising analytical results.

3- Developing services to laboratories :

- ⇒ Service-ICAR could be recipient of analytical service requests from ICAR members (e.g. analytical information, teaching, auditing, reference materials, proficiency studies) and orient them toward specialised organisations by proposing a list of service suppliers and internet links.
- ⇒ Reference system : ICAR Reference Laboratory Network appears as the most appropriate tool to define consensual reference values for international Reference Materials to be used either in reference methods checks or in calibration of routine methods in connection with international

interlaboratory trials. RMs characterisation activity can derive in the future from the development of new guidelines already mentioned above.

4- Optimising the efficiency of the list of reference laboratory :

Development of a laboratory data base for ICAR on the Internet that can provides ease in connection and information and be a medium for presentation and search to external observers and benchmarking. Larger developments can be foreseen such as in-line questionnaire for international surveys in analytical issues, automated search of service suppliers or contacts for technical issue, etc.

Conclusion

The analytical quality assurance system of ICAR is a major tool for ICAR and will keep topical since milk recording and genetic evaluation are international issues today. Its maintenance and development is an issue for ICAR. With this respect, new objectives can be proposed to MTL WG for the next years.

References

Baumgartner c., Landgraf A., (2005): Deep frozen raw milk standards. The way from reference methods to reference systems. Proceedings of 34th Biennial Session of ICAR. Sousse, Tunisia, May 28-June 3, 2004. Performance Recording of Animals. State of the art, 2004. EAAP publication n°113, 2005, 253-257.

Leray O., (1995): Contrôle qualité et harmonisation des pratiques dans les laboratoires laitiers : Un enjeu pour le contrôle laitier / *Quality control and harmonisation of laboratory practices in dairy laboratories : A challenge to milk recording*. Proceedings of 29th Biennial Session of the International Committee for Animal Recording (ICAR). Ottawa, Canada, July 31-August 5, 1994. EAAP publication n°75, 1995, 179-182.

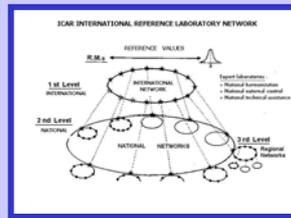
Leray O., (1998): Quality control of conventional mid-infra-red milk analysers using recombined milk samples. Proceedings of 31st Biennial Session of the International Committee for Animal Recording (ICAR). Performance Recording of Animals. State of the art, 1998. Rotorua, New Zealand, 18-23 January, 1998. EAAP publication n°91, 1998, 131-138.

Leray O., Trossat P., (1996): Calibration and quality control of automated somatic cell counters using recombined milk samples. Proceedings of 30th Biennial Session of the International Committee for Animal Recording (ICAR). Performance Recording of Animals. State of the art, 1996. Veldhoven, The Netherlands, 23-28 June, 1996. EAAP publication n°87, 1996, 197-200.

ICAR AQA Strategy

Implementation and prospect

Olivier Leray, Cecalait, France



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ICAR AQA system presented in Ottawa 1994 ⇒ Start up

Objectives :

- 1- Definition & characterisation of **appropriate** analytical methods
- 2- Definition of **minimum conditions** of quality assurance (methods, samples, control)
- 3- Definition of **a frame and a model** for harmonising and structuring AQA
- 4- Definition of **tools** to achieve lab harmonisation and establish analytical traceability

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First period of implementation (1994-2006)

Completion of the first objectives of Ottawa :

1- Definition & characterisation of appropriate analytical methods:

- ⇒ **IDF/ISO standardisation** (revision & creation : F, P, L, urea, SCC, sheep & goat)
- ⇒ **ICAR protocol** for the evaluation/approval of routine methods (2002)
- ⇒ **Approval process** implementation (July 2006)

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First period of implementation (1994-2006)

Completion of the first objectives of Ottawa :

2- Definition of minimum conditions of quality assurance (methods, samples, control) :

- ⇒ **Guidelines for QA in milk recording analysis:**
 - Circulation for use in 1998
 - revision in 2006
 - publication in ICAR guidelines in 2006

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First period of implementation (1994-2006)

Completion of the first objectives of Ottawa :

3- Definition of **a frame and a model** for harmonising and structuring AQA (1996):

- ⇒ **International network** of reference laboratories
- ⇒ **National routine lab monitoring** by reference laboratories
- ⇒ **List of competence = Missions (suggested) = Eligibility criteria to the network**

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First period of implementation (1994-2006)

Completion of the first objectives of Ottawa :

4- Definition of **tools** to achieve lab harmonisation and establish analytical traceability:

- ⇒ **Proficiency testing schemes :**
 - Standard protocol and standard data treatment (from 1996)
- ⇒ **Reference materials:** (publ. in ICAR Session proceedings)
 - Recombined milk samples for calibration or control for composition (MIR) and SCC (Leray, 1990, 1996, 1998)
 - Long term preservation by deep-freezing (Baumgartner, 2004)

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Second period of implementation (from 2006)

1- A new way of life for national organisation?

ICAR QA policy oriented towards **Quality Certificates** and
Special Stamp granting

Thus for milk analysis and laboratories

strengthening and **formalising** involvement of ICAR national
organisations in AQA

since then ...

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Second period of implementation (from 2006)

Whereas

First period = **Invitation** to ICAR members to enter the AQA
system proposed by ICAR and use it for own QA system
(accreditation)

Second period = **Incitation** to ICAR members to follow ICAR
guidelines and take part regularly in laboratory network
proficiency testing **as prerequisite to benefit of ICAR
quality certification**

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Second period of implementation (from 2006)

- ⇒ **Nomination** : Every dairy country of ICAR should nominate **a minimum of one laboratory** so as to enable **linkage** between national and international levels
- ⇒ **Harmonisation & traceability** : Every dairy country of ICAR should involve **a minimum of one laboratory in each trial** of the two-yearly ICAR proficiency testing scheme so as to establish the effective **consensual analytical truth** for ICAR

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Second period of implementation (from 2006)

2- Enhancing harmonisation in laboratory practices = Improvement of the toolbox

- ⇒ **Standard protocols (guides) for reference laboratories** :
 - **Proficiency testing** : Harmonised protocol to enable comparison between countries
 - **Centralised calibration** : Guide for experimentation, elements of decision and calibration samples, organisation & implementation

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Second period of implementation (from 2006)

3- Developing AQA services to laboratories :

- ⇒ **Service ICAR :** (Hypothesis of work)
Recipient/intermediate for analytical service requests of ICAR members (e.g. analytical information, teaching, auditing, reference materials, etc)
and orient them to specialised organisations ⇒ list of service suppliers on the web site and internet links.
- ⇒ **ICAR ref lab network :** Characterisation of RMs (reference values) in a Reference system.

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Second period of implementation (from 2006)

4- Optimising networking :

- ⇒ **Searchable data base on laboratories in ICAR website :**
- 1- Presentation of the members **ICAR Reference Laboratory Network**.
 - 2- Possibility for a **complete presentation of routine laboratories in ICAR member countries** through individual web spaces dedicated to countries in connection with the network member list.

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Conclusion

The AQA system of ICAR is a major topical tool and will keep being in the future since milk recording and genetic evaluation are international issues today.

Its maintenance and development rest an issue for ICAR.

With this respect, new objectives can be proposed to MTL WG for the next years.

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Thank You for your attention!

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Policies of ICAR about Milk Analysis Aspect

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The importance of milk analysis

In the very early years of its existence, the International Committee for Animal Recording (ICAR) was called “European Milk and Butter Committee”. The current name, ICAR, was assigned after many years of activity. It was clear, since the beginning, that the policy of ICAR was concentrated on milk analysis, first regarding fat percentage and later protein and other milk constituents. As the economic value of milk constituents became more important, the value of milk analyses increased. In the first years of ICAR’s history, fat was very important also as a selection goal. When the economic conditions changed and so did human dietary requirements, protein increased in importance and became more valuable, in terms of selection goals, than fat percentage. Milk constituents (mainly fat and protein) were analysed not only for selection purposes, but also in relation to payments to farmers. Milk was paid in proportion to the percentages of fat and protein, thus leading farmers to consider the importance of milk analyses as crucial. Cheese production was also largely influenced by milk quality, in terms of fat and protein quantity and characteristics. Cheese industries developed, since the very early years, the possibility to detect the quality of milk for a more efficient utilisation during the milk processing activities. The importance of other constituents, like somatic cell count, urea, etc, increased in the last years as well.

The importance of Milk Analysers Test

The development of instrumental analytical methods followed the increasing needs of breeders, breeders’ organisations and cheese industries. The economic efficiency of milk analysers has increased considerably, from about 20/40 analyses/day/person a few decades ago to currently 500 analyses/hour/instrument. This development was made possible only by maintaining or improving the precision of milk analysers. To check the accuracy and the reliability of milk analysers, tests were developed to assess performance characteristics and practicability for laboratories. In the last years, breeders’ organisations stressed the need for homogeneity of performance among laboratories producing data for genetic evaluation. For this purpose, ICAR recently developed a harmonisation protocol for milk records.

The ICAR working group of milk testing laboratories produced detailed requirements for the harmonization protocol. The proposal, accepted by the Board, was to grant worldwide valid approval of instruments/methods. The documentation explaining the methods and details for harmonisation was developed and submitted in 2002. ICAR governing and technical bodies had been discussing this document for long, especially in the 2004 biennial meeting held in Sousse (Tunisia). The Board approved it in 2006 and submitted to the General Assembly that gave its positive opinion. Following such decisions, as from 1st July 2006, the protocol of testing and consequent approval of milk analysers became effective. The approval can be requested by manufacturers or by any ICAR member organisation. National approvals are essential to gain the final ICAR approval; in fact, approval by three different countries is necessary for this purpose.

The importance of harmonization of laboratories performance

ICAR’s policy has always been directed at the dissemination of information and experience in milk recording, animal identification and genetic evaluation. Another main objective of ICAR is the harmonisation of such systems, including those of milk recording. For this purpose, the milk testing laboratories working group has been a great source of technical standardization and of exchange of information and expertise among laboratories. For dairy animals, the most important genetic indexes, essential tool for genetic improvement, are composed by the genetic values of protein and fat percentage. Since the genetic indexes of sires are compared by means of Interbull indexes, the data utilised to form those indexes coming from all ICAR member countries should be taken with similar and comparable methods. This is true for data collected directly in the farm and for the data coming from milk laboratories.

The working standards of the laboratories must be excellent. The relative ICAR working group has always been effective, in all its historical activities, in helping every laboratory participating in the network to improve their performance. The accuracy of milk constituents analysis has been improved in the last years thanks to the international technological improvements and the improvement of technicians' skills. Both these improvements were pushed by higher expectations from farmers and industries than in the past.

From Working Group to Sub-Committee

ICAR is organised in such a way that the objectives of its working groups are directed at specific and not perpetual tasks. On the other hand, the objectives of the ICAR Sub-Committees are related to permanent services for members. The current ICAR Sub-Committees are: i) Identification, ii) Interbull and iii) Recording Devices. The ICAR Board, considering that the milk testing laboratories working group is already offering permanent services to ICAR members, proposed to the General Assembly the status of Sub-Committee for the working group. This decision was also taken considering that the Sub-Committee will have to provide more services in the future.

From Special Stamp to Quality Assurance: reasons for change

ICAR has just approved a modification in the statute, allowing new membership profiles. The members' requirements are also developing, becoming more and more interested in the economic efficiency of their service. What is more, ICAR's strategy became more focused on quality assurance for its members.

One of the most appreciated ICAR services is the Special Stamp visit, during which ICAR experts give advice and certify the technical standards of members' services. A sharp restructuring of the Special Stamp visit was planned, and the new service was named Quality Assurance. The Quality Assurance is aimed at maintaining ICAR standards and is more dedicated to an efficient advisory to improve the relevance of members' services.

In relation to the new service, a panel of ICAR auditors will be created for performing the test on quality assurance. The auditors must be experts and appointed by the ICAR Board, while the latter will issue the quality assurance certificates after reviewing the report of the auditors who performed the visit. The quality assurance certificates will be valid for three years. The on-site inspections will be carried out in alternate certified periods, i.e. mandatory auditor visit every six years; in the meantime the member might provide appropriate documentation to guarantee the respect of the level of accuracy and efficiency of its service so to keep the quality certificate. This is a paid-for service. Those countries that already hold a Special Stamp will have time until 2009 to request a quality assurance visit.

The auditor visits will be requested by member organizations for specific activities and species. ICAR has planned three types of services: identification, performance recording and genetic evaluation, and five different species or production systems: dairy cattle, beef cattle, sheep, goats, and buffalo. The auditors will offer advice and check members for the application of guidelines and the quality of provided services. To achieve high efficiency in this specific service, the guidelines must be constantly updated. The Sub-Committee will constantly update the guidelines according to technical and organizational evolution. Therefore, as a result of the Quality Assurance service, the activities of Sub-Committee of Milk Testing Laboratories will now include the constant update of ICAR guidelines, the detailed definition of the minimum acceptable standards, and the nomination of experts to include in the list of auditors.

The future

ICAR will have to plan, together with the Sub-Committee on milk testing laboratories, new developments to improve the efficiency of services to its own members. Some possible developments can be defined:

A network

ICAR is interested in creating a network of milk laboratories. The network must be structured on several levels. The first level comprises national – or institutional/company – reference laboratories whose tests are harmonised by the ICAR International Ring Test. The second level is composed of local laboratories that are harmonised through a Ring Test organised by a national or local reference laboratory

participating in the first level of Ring Test. There will possibly be a third level of Ring Test, or even more, if it is necessary for reaching the important objective: all laboratories working for ICAR member organizations must form a network so that the harmonisation of procedures is feasible for every performance recording structure.

New analyses

The development of animal industries requires the possibility to measure new traits, including new milk constituents, for a wide range of objectives. One objective with increasing importance in the last decades is the possibility to obtain more information for herd management purposes. The test of many new constituents of milk is used to decide about individual or group feeding and to facilitate other decisions regarding feeding animals, culling, breeding decisions, etc. Some cows' metabolic diseases can be in fact detected by some new tests. The milk process industries require new tests to improve decision-making about the raw material to process. The presence of drugs and other abnormal constituents in milk must be considered to enhance the value of milk analysis. For all these activities, the Sub-Committee must set up new standards for Ring Tests and for milk analyzer approval tests.

In-line analysers

A new technology has been developed by some milk analyser industries: the in-line analysers. This system will allow analysing milk directly in the farm for every individual cow at every milking. The revolution to be expected by this new technology will have certainly a great impact, when the technical problems still to solve will be sorted out and if it will be available for a large number of farmers. Nowadays, this system is still in the development stage – although quite advanced. Commercial diffusion of in-line analysers has not taken place yet. There are some technical issues to resolve, like the calibration of the in-line analysers for detecting some particular traits, like somatic cell count. The ICAR Sub-Committee is committed to set up guidelines and standards for the in-line analysers. The definition of the standards and guidelines must be done in co-operation with other Sub-Committees and working groups for there are specific techniques in the entire system that are relevant to identification and milk yield measurement. For this purpose it might be more efficient to develop, in the near future, the "entire herd management system test".

Information network

The ICAR website had been receiving an extraordinary number of visits. During the last year, there have been about 100000 visits. The website is organised in such a way that every group has its own page for spreading information about activities, developments, meetings, new guidelines and standards, contacts, etc. The website can thus be a very important tool for exchanging technical information. For example, many visitors of the "Identification" and "Recording Devices" pages look directly for the list of the relative approved devices. The same can be done for approved milk analysers in the already existing web pages dedicated to milk testing laboratories. There can be information for and about milk laboratories participating in the Ring Tests. The participation of private industries might also be asked. Furthermore, the website page could present and propose books, proceedings, technical articles, meetings and workshops. To enhance their importance, these pages could be enriched with job postings, announcements, people facts and other information useful for those interested in milk analysis activities.

Large farms

The global changes in dairy cattle farms highlight an evident increase in the average herd size. This trend is more visible in the USA and in the most economically and technological advanced countries. Breeders' organisations must be prepared to offer appropriate services for large farms managed like standard private companies and not like family business anymore. The types of services required by the large farms are connected to new and efficient milk analysis technologies. Breeders' organisations must be fast in giving answers, the price per single analysis must be low and it must continue to be reasonable. Since workers do most of the activities in the large farms, the sampling collection must be easy. Some large farms might consider it convenient to install in-line analysers, wherever available, or to have in-house a

laboratory. In the latter case, the breeders' organisation must perform the service of harmonisation of instruments and, through a lower level of Ring Test, maintain a minimum of working standards.

Long-term vision

It is ICAR's responsibility to have a long-term vision of all its activities. For its importance in the ICAR fields of operation, the activities of the milk testing laboratory Sub-Committee is among those who will require special commitments to plan future activities. A new possible field of operation could be the DNA analysis from milk samples. This new type of DNA analyses might provide information about cows' genotypes, and therefore defects, production levels, disease predisposition, etc. To perform this type of service, joint actions with DNA laboratories are required. DNA analysis on milk can be an important tool for food traceability as well as for cow identification.

Another interesting long-term vision of possible activities is the detection of specific residuals. Milk analyses could be used as an environmental detection tool, since some polluting elements pass through the cows, from animal feed directly to milk. Such a service is expected to instigate the public interest. For the same objectives and through similar methodologies, milk analyses can be utilised as food safety sentinels.

Policies of ICAR about Milk Analysis Aspect



Andrea Rosati

The Importance of Milk Analysis

- Importance since the beginning (European Milk and Butter Committee, 1951)
- Economic value of milk constituents
 - Difference between Protein and Fat
 - Payment to farmers
 - Genetic indexes
 - Cheese making industries
 - Other constituents



The Importance of Milk Analyzers Test

- Development of instrumental analytical methods
- From about 20/40 analysis/day/person to 500 analysis/hour/instrument
- From:
 - test only to assess performance characteristics and practicability for laboratories
- To:
 - harmonization protocol for milk records purposes



The Importance of Milk Analyzers Test

- Detailed requirements for harmonization protocol
- Proposal from the working group MTL to grant world wide valid approval of instruments/methods
 - Documents submitted on 2002
 - Support in the Sousse biennial meeting (2004)
 - Board approval on 2006 - Submission to the General Assembly
 - Beginning on 1st July 2006



The Importance of Milk Analyzers Test

- Request by manufacturers or by any ICAR Member Organization
- National approvals
- Three different countries approval



The Importance of Harmonization of Laboratories Performance

- ICAR efforts for harmonization of milk recording
- The MTL has been a great source of harmonization and of exchange of information and expertise among laboratories
- The importance for genetic evaluation



High Laboratories Performance Working Standards

- ICAR MTL working group activities help every laboratories participating to the network to improve their performance
- The accuracy of milk constituents analysis has been improved in the last years due to:
 - technological improvements
 - improvement of technicians' skills
 - higher expectations from farmers and industries



From Working Group to Sub-Committee

- ICAR working groups objectives are finalized to a specific and not perpetual tasks
- ICAR Sub-Committees objectives are related to permanent services for members
- Actual Sub-Committees are: Identification, Interbull, Recording Devices



From Working Group to Sub-Committee

- ICAR MTL working group is already giving permanent services to ICAR members
- More services are planned for the future
- Board decision to be endorsed by the General Assembly



From Special Stamp to Quality Assurance: Reasons for Change

- Changing membership profile
- Members changing requirements
- Greater emphasis on visible quality assurance building on ICAR's reputation
- Maintaining ICAR standards
- Relevance to members' actual activities



Process

- Certificates will be valid for 3 years
- A panel of ICAR auditors
- On-site inspection for alternate certified periods
- Prompt feedback to members
- Paid-for service
- 2009



The Impact on the Laboratories Activities and Services

- Auditors' visits requested by member organizations for specific activities and species:
 - identification, recording, genetic evaluation
 - dairy cattle, beef cattle, sheep, goats, buffalo
- Auditors' visits will advise and check on guidelines application
- The Sub-Committee must constantly update the guidelines to technical and organizational evolution



The Future: a network (1)

- Creation of a network of labs based on several levels:
 - 1st level: national (or organizational) reference laboratories harmonized by the ICAR International Ring Test
 - 2nd level: local laboratories harmonized by national (or organizational) Ring Tests
 - 3rd level: ...
- Objective: every laboratories working for ICAR member organizations must be in a network



The Future: new analyses (2)

- New traits, or milk constituents, for large variety of objectives:
 - herd management
 - cheese industries
 - cows metabolic diseases
 - cows nutrition
 - drugs presence
 - ...
- New standards for Ring Tests and for milk analyzers approval tests



The Future: In-Line Analyzers (3)

- New technologies for in-line analyzers
- Commercial diffusion of in-line analyzers
- Calibration of in-line analyzers
- Somatic cells counts issue
- Checking of working standards of in-line analyzers
- Joint activities with Recording Devices SC
- Entire herd management system test



The Future: Information Network (4)

- Full use of the MTL pages in the ICAR website:
 - guidelines
 - exchange of technical information
 - information about approved milk analyzers
 - milk laboratories participating to the Ring Tests
 - Participation of private industries
- Books, proceedings, technical articles, meetings, workshops, ...
- Job posting, announcements, people facts, ...



The Future: Large Farms (5)

- New and efficient milk analysis technologies for large farms:
 - fast
 - cheap
 - relevant
 - immediate response
- Easy farmers sampling collection
- In-house laboratory:
 - harmonization of instruments and working standard (lower level Ring Test)
 - service to farmers



The Future: Long Term Vision (6)

- DNA analyses from milk samples:
 - Information about cows genotypes (defects, production levels, disease predisposition, ...)
 - Joint actions with DNA laboratories
 - Food traceability
 - Cow identification
- Detection of specific residuals:
 - environmental detection tool
 - food safety sentinel



Summary

- Milk analyzers test
- Harmonization of laboratories standard
- From working group to Sub-Committee
- From Special Stamp to Quality Assurance
- The Future:
 - network
 - new analyses
 - in-line analyzers
 - information tools
 - large farms
 - the way beyond



**Thanks for Your
Attention!**

Reference System – Principle and Practice

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Abstract

The definition of “reference” covers two fields of meaning. One is dealing with “testimonial”, “endorsement” or “certification”; the other involves the aspect of “information”, “evidence” or “source”. These definitions describe very well the intentions of reference systems, which shall replace the traditional way of calibration of routine methods.

Traditionally linear calibration models are used, linking one routine analyzer via the characterisation of a calibration material to the reference method, carried out in a reference laboratory. This traditional scheme underlies some major restrictions: insufficient definition of the parameter to be measured, poor precision of reference methods compared to the precision data of routine methods, high variation between reference labs carrying out the reference methods, stability and homogeneity of characterized calibration materials (secondary reference materials – SRMs) and others.

But anyhow, due to the demands of a globalizing trade, analytical results should be comparable and equivalent worldwide, over time on the long run and between different analytical methods. This asks for more intelligent reference systems, using all information to control all sources of variation in the whole analytical process. Thus, feed back mechanisms can optimize and reduce the variation in carrying out reference methods as well as prove the validity of the SRMs used. Using the redundancy of the calibration data of routine analyzer clusters, much more information could be obtained about the quality of SRMs and their target values as well as the quality of routine calibrations.

To set up such a reference system a centralized data base has to be created to gather calibration data of routine analyzers. Of course a reporting system for routine labs is as crucial for the success as a reporting and cooperation system for the reference labs. As far as legally important and controlled parameters are concerned, also the competent authorities and scientists have to be part of such a reference system to assure an internationally accepted reference level for the respective parameter.

Up to now, the international structures to implement such a reference system are missing. ICAR should define its role and how to act to become a part of such international analytical reference systems.

Keywords: Baumgartner, reference, calibration, routine method, secondary reference materials;



MDP
Milchprüfung Bayern e.V.



Reference System Principle and Practise

Meeting of ICAR Reference Laboratory Network

35th ICAR Session
Kuopio, Finland
6 June 2006



MDP
Milchprüfung Bayern e.V.

Contents briefly...

- What does „reference“ mean?
- „reference“ in a linear calibration model
- draw-backs → „reference system“
- an ideal reference system for SCC as an example
- some conclusions

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Reference?

- Definition 1 → **testimonial**
 Synonyms = certificate, certification, character, endorsement, contribution, recommendation, tribute
- Definition 2 → **information**
 Synonyms = dictionary, encyclopedia, evidence, source, thesaurus, writing

reference manual
 reference method
 reference material

Roget's New Millennium™ Thesaurus
<http://thesaurus.reference.com>

Milchprüfing Bayern e.V.

Reference – the traditional way of life

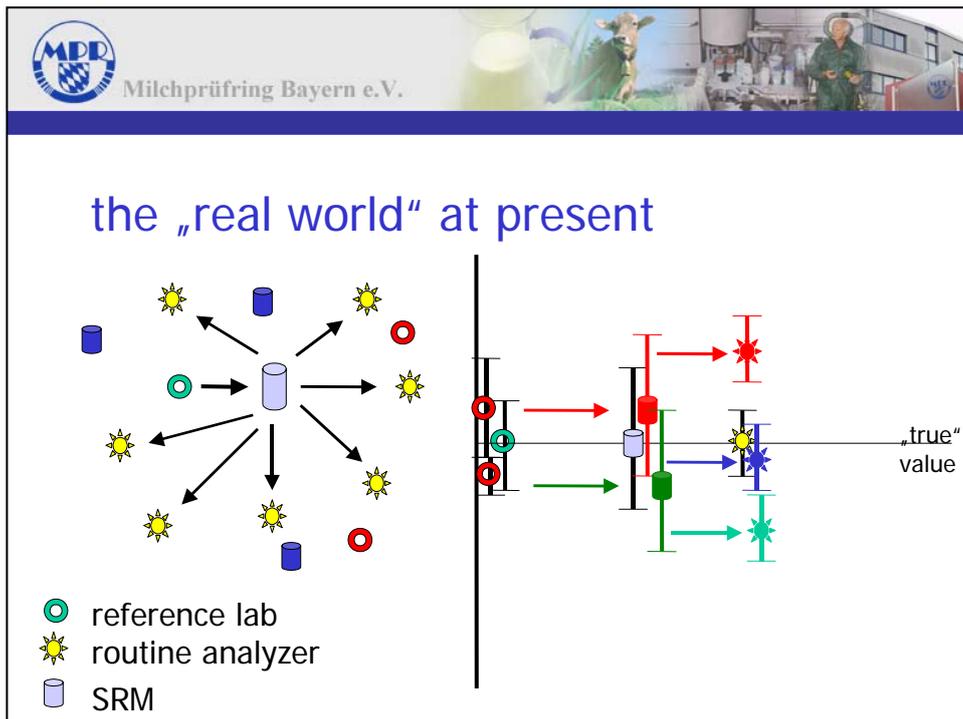
ref.me1 24.000 → SCC

„linear calibration model“

calibration
 relating something variable to a certified source

reference lab
 routine analyzer → source of limitation
 secondary reference material

ref.met = reference method




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Why do we need different methods?

- reference methods for definition
- routine methods for “daily life”
- routine methods are often automated methods because of
 - high throughput
 - high performance (precision characteristics)
 - data availability and handling
 - low labour, low costs



Routine raw milk testing

	Germany	World*
Labs	18	700 (1.700)
Samples/year	40 Mio.	300 – 500 Mio.
Analyses	140 Mio.	1.000 – 1.300 Mio.

* roughly estimated



linear calibration model → reference system

Analytical results should be comparable and
“equivalent” ...and they are not!!!

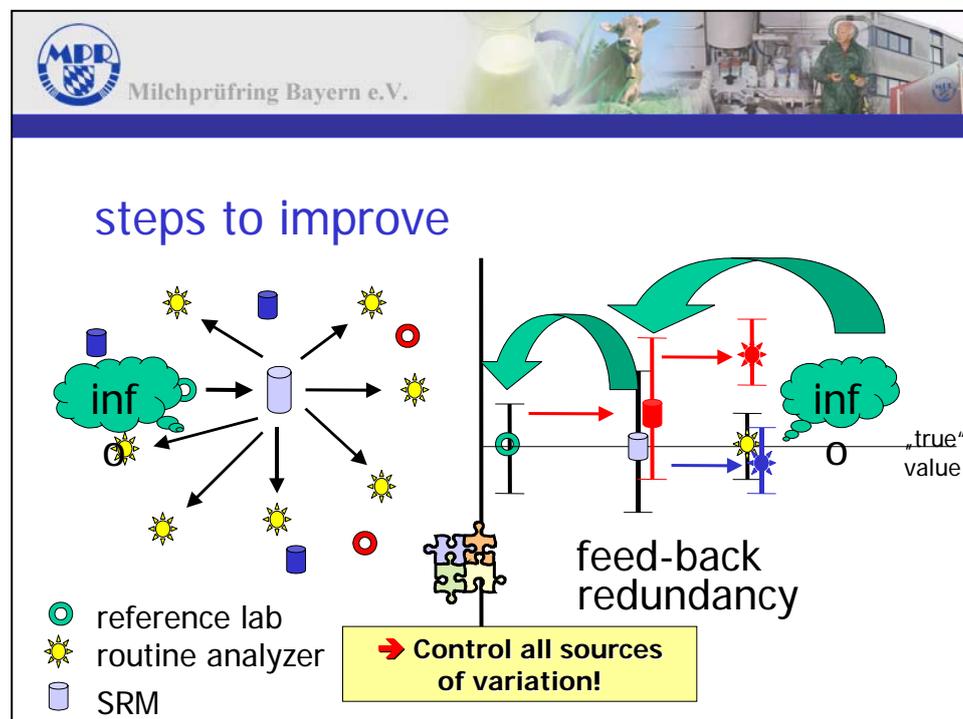
- worldwide
- over time on the long run
- between different methods

→ anywhere – anytime – anyhow

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Main limitations of traditional calibration schemes

- ⇒ there is an insufficient definition of the parameter to measure ("definition of the truth")
- ⇒ the uncertainty of analysis linked to the reference method is high
- ⇒ there is no CRM which could be used as a "golden standard"
- ⇒ the use of available SRMs is limited by shelf life and poor homogeneity of batches during the labeled shelf life





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Ideal system for somatic cell counting

- ⇒ a clear definition, what SCC is (which cell populations to be counted, ...) → what do we want to know?
- ⇒ a precise (highly repeatable/reproducible) standard or "reference" method, based on clear precision data from method evaluation AND practical use
- ⇒ a well defined certified reference material (CRM) and several secondary reference materials (SRMs) based on different types of (milk) cell populations for calibration purpose with high stability over a very long shelf life to be used in routine raw milk analysis
- ⇒ high-yielding SCC analyzers for routine applications



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Ideal system for somatic cell counting

- ⇒ a reporting system for calibration data of all routine SCC analyzers
- ⇒ a data base to collect all data and to provide information for routine AND reference labs
- ⇒ a network of labs performing the reference method as anchors to fix an internationally accepted SCC level
- ⇒ a network of "safeguards" to provide all necessary input to the system from all parties involved (competent authorities, reference labs, routine labs, scientists and "users" as breeding and DHI organizations, dairy companies etc.) and to develop the system to its best function.



Implementation – status report

- 😊 Step 1: reference method – definition of “the truth”
- 😐 Step 2: CRM (???) and SRMs (officially adopted)
- 😞 Step 3: proficiency testing schemes
- 😞 Step 4: reference lab network
- 😞 Step 5: training courses
- 😞 Step 6: data collection and extracting
- 😞 Step 7: developing procedures for “interference”- how should elements of the system influence each other
- 😞 Step 8: safeguarding network (competent authorities, reference labs, routine labs, scientists and “users” as breeding and DHI organizations, dairy companies etc.)



Conclusions

- Instead of single linear models „reference systems“ are needed, using feed back and redundancy effects.
- All systems should be interlinked, to utilize all information available!
- International structures for implementation are missing so far. What ist the role of ICAR?
- **We could do much better as we are doing now!**

Reference and calibration system for routine milk testing – Advantages / Disadvantages, Choice criteria

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Introduction

Optimising the overall precision of analytical results is the constant concern of analysts. The choice of the analytical system – instrument, calibration techniques, quality control – derives from a compromise between the cost of implementing and running the system and the benefit pertaining from the precision of results.

In milk recording, automated infra red milk analysers are the most commonly instruments used for routine testing in milk composition. As alternative methods, those instruments need to be calibrated using standardised reference methods whatever the criteria according to relevant standard in line with ISO 8196 | IDF 128.

Thereby overall precision is conditioned by both the precision of reference method used for calibration and the overall accuracy of milk analysers such as defined in ISO 8196 | IDF 128 : precision (repeatability and reproducibility), exactness of calibration and accuracy.

The task of the laboratory is then to strive to minimise errors related to both the reference and the routine method through appropriate choices in the reference and calibration systems applied.

Reference system

A reference system refers to the way used by a laboratory or a group of laboratories to obtain reference values relevant to calibrate routine methods. It is possible to distinguish between different system with increasing degree of security and confidence

- Individual laboratory alone: At the beginning is the individual laboratory - autonomous but less organised system - for which a tight observance of method standards is the only safeguard and reference method performance expected at least the standard precision of the method. Such a basic system does not prevent the laboratory from possible discrepancies resulting involuntary deviations due to the specific local situation (i.e. chemicals, practice or instrument). This case prevailed before the advent of the implementation of analytical quality assurance (AQA) and accreditation.

- Laboratory working under quality assurance : AQA introduces the fundamental point of getting an external view of the laboratory and neutral external performance evaluation. By participating regularly in proficiency testing and using external reference material allow the laboratory to establish links with other sources of reference and improve its performance with the method. Indeed, consensual reference values established by numerous laboratories show lower uncertainties hence are admitted as closer to the unknown true values. Thus it is worth to refer to those values to detect misuse of the method and move to optimising. This system is the most generalised up to now.

- Laboratory in a centralised calibration system : It is based on the same principle that higher trueness and confidence in reference values are better guaranteed when obtained by several laboratories. Interlaboratory studies a central organisation are organised in order to determine reference values for a set of calibration sample batches through the average values of the group of participants. It can be combined with the previous system so as to make the lab benefit from both the external evaluation of the reference method and calibration facilities. In that case, all the labs contribute in establishing the reference. Alternatively it can be performed by a sub-group specifically identified so-called reference laboratories.

Calibration system

A calibration system refers to the way used by a laboratory or a group of laboratories to evaluate the proper relationship linking the instrumental response and the true value obtained by the reference method for the population currently analysed. Calibration operation is time consuming and requires skill and experience as made of numerous technical steps :

- select samples to be collected to assure representativeness and sufficient component ranges,
- carry out sample collect and perform the production/preparation of calibration samples,
- perform analyses by the reference methods,
- perform analyses by the routine analysers,
- proceed to the statistical analysis of results, evaluate the current calibration line, adjust calibration and define new values of pilot samples.

If the two latter steps are entirely part of the laboratory competence, the three first ones can be either performed locally by the laboratory for its collect area or, at a larger scale, by a single organisation/laboratory for a wider area including several laboratories. This is defined as centralised calibration by opposition to local calibration which allows to consider calibration from two points of view, individual or collective.

In the principle, the system that involves centralised calibration offers the maximum security by optimising calibration sample characteristics (e.g. component range, physicochemical quality and preservation) and can be also the source of great economy by reducing drastically the overall calibration costs and sharing between laboratories.

Analytical system

Associating the different possibilities of reference systems and calibration systems give room to different combination of possible analytical systems of various degree of interest with regard to security, ease to laboratories and overall cost. It comes to evidence that a collective analytical system combining a collective reference and a centralised calibration is an optimal situation whereas the situation of the isolated laboratory is the poorest.

Combination	Reference	Calibration	Precision of reference	Representativeness	Component range
1	In-house	In-house	Possibly over standard	Optimal	Variable
2	In-house + PTs	In-house	Standard	Optimal	Variable
3	In-house + RMs	In-house	Improved	Optimal	Variable
4	Reference laboratory	In-house	Standard	Optimal	Variable
5	Reference laboratory	Centralised	Standard	Acceptable to optimal	Optimal
6	Laboratory network	Centralised	Optimal	Acceptable to optimal	Optimal

Nevertheless the availability of some of the combination are directly related to the routine methods used and/or the natural factors that introduce heterogeneity and variability in milk composition.

State of the art in mid infra red measurements

Technical tools have been developed since the eighties to optimise sample calibration preparation (Leray, 1998) and their preservation (Baumgartner & al, 2004).

Nevertheless, a centralised calibration is generally applied where the method response is not (or weakly) dependent of the milk analysed. Indeed, routine method sensible to matrix effects can show differences between different region or collect areas due to different milk composition then it is to put in balance advantages (pros) and disadvantages (cons) before making the final choice.

Well known interferent effects, that cannot be corrected in classical filter analysers and can inflate the accuracy for a population with heterogeneous composition of milk, are :

- for fat, the fatty acid composition of milk fat, stronger at 5,7 μm than at 3,5 μm measurement wavelengths and in the opposite direction and sensitivity to unsaturated fatty acids at 3,5 μm .
- for protein, carboxylic acids, mainly citric acid.

Whereas, lactose is generally measured and used to improve the estimate of fat and protein.

Their variation are related essentially to feeding and metabolism in tight relation with natural local food sources (pastures, ensilages, etc) or by-products of local food industries (beat pulp, etc), all of this being summarised in the wording seasonal and geographical effects.

Since the advent of mid infra red interferometers with Fourier Transform the whole spectrum is now available for measurements. Wavelength specific to double bounds for fat, urea and citrates for protein have brought the possibility for more intercorrections with optimised multivariate regression model (PLS) and it is likely the situation noted with classical filter instruments has been improved in a large extent.

Situation with classical filter devices

Existing cases have been studied in the past at the national level of France (Leray, 1989) and at the European level through a BCR Programme (Grappin & al, 1994) with regard to mid infra red fat, protein and lactose determinations.

Already in France, a centralised calibration was found appropriate for fat 3,5 μm - maximum range between region/lab and regional accuracy standard deviation of 0,06 and 0,05 % fat respectively - and for true protein - maximum range between region/lab and regional accuracy standard deviation of 0,02 and 0,03 % true protein respectively -, whereas stronger influence can be observed for fat 5,7 μm at diet change periods (Nov. 1981) - maximum range between region/lab and regional accuracy standard deviation of 0,10 and 0,08 % fat respectively - in accordance with older studies.

BCR European Programme confirmed the former observations with regard to the influence of interferents and indicated that true protein (TP) could be used at a large scale in Europe whereas fat at 5,7 μm and crude protein (CP) showed larger range of mean calibration biases over a period of one year. At the European scale, fat 3,5 μm did show much lower range between countries than with fat 5,7 μm (0,071 versus 0,104 % fat) and lower local accuracy standard deviations (0,059 versus 0,053 % fat). Similarly True Protein showed much lower range between countries than with Crude Protein (0,077 versus 0,048 % protein))and also lower local accuracy standard deviations (0,046 versus 0,032 % protein). Furthermore the adequacy of recombined calibration samples (Leray, 1988, 1989, 1990, IDF 141:1991-2000) was assessed and confirmed for as large areas as Europe with regard to slope and intercorrection fitting.

Choice criteria

The overall error of measurement is composed of error of the reference method and the error of accuracy of the routine method at the mean level for the laboratory or the relevant collect area. Centralised calibration is up to minimise the reference method error against a possible increase of local biases resulting from a loss of calibration sample representativeness for every part of the broad collect area (several laboratories). Thus :

Choice for the routine method

This should be for the one with the lowest sensitivity to matrix effects, thereby, for mid infra red as the method used worldwide today, preferably applying rank of increasing suitability:

- for fat : fat 5,7 μm , fat 3,5 μm and fat by interferometry with Fourier Transform on the whole mid infra red spectrum (FT-MIR),
- for protein : Crude Protein 6,5 μm , True Protein 6,5 μm , protein (CP or TP) by interferometry with Fourier Transform on the whole mid infra red spectrum (FT-MIR).

Choice for the calibration system (local vs centralised)

It can only be made through an appropriate experiment (Figure 1) that allows to gather sufficient amounts of analytical data (reference and routine methods) obtained on sample representative of the whole region to be submitted to the centralised calibration and throughout a full year period to account the various animal feeding situation. Numbers of region, samples, replications are to be determined so that minimum systematic bias that are accepted should be statistically significant. Classical treatment by analysis of variance and usual statistics leads to determine whether or not possible effects observed are acceptable with respect to the purpose of analyses then to adopt or not such a centralised system.

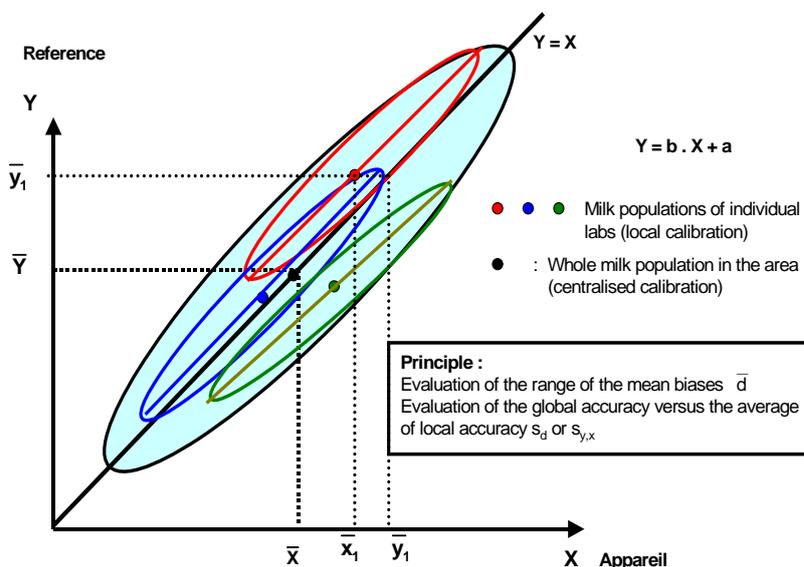


Figure 1. Example of experiment - Parameters and principle for assessing the possibility of centralised calibration

Otherwise a pragmatic approach based on proficiency testing (PT) studies can provide a proper indication on how far a centralised system would help in more analytical harmonisation. Comparing PT results with reference method and those of PT with routine methods calibrated with the former reference method for a group of laboratories covering the same or similar collect area can show whether or not regional effects (matrix) are effective or not (Figure 2). Much larger distribution of systematic bias with routine methods would indicate likely significant regional effect whereas similar distribution width would plea for an possible alternative by centralised calibration. Special case of smaller distributions with routine methods would rather indicate part of laboratories have already reduced the error of reference method (possibly by centralised calibration samples) which is observed in the absence of matrix or regional effects.

Conclusion

Where applicable, centralised systems for reference and calibration appear being more convenient tools for laboratories and more securing systems for users

Centralised calibration requires either routine methods insensitive to matrix effects or to concern areas with negligible variation in matrix composition. Otherwise its applicability relates to the loss of precision accepted compared to the advantages of a centralisation of the reference and to the uncertainty of analytical results needed by users.

Tools for the application of centralised calibration systems already exist and are published in ICAR Sessions proceedings that are appropriate method for calibration sample preparation (RMs), amans for mid (chemicals) and long term (deep freezing) preservation, and structure to check or determine reference values (ICAR Reference Laboratory Network).

Moreover centralised calibration can be also a general answer to the question of checking/fitting calibration of in farm analytical devices which appears to be the next challenge of the forthcoming years.

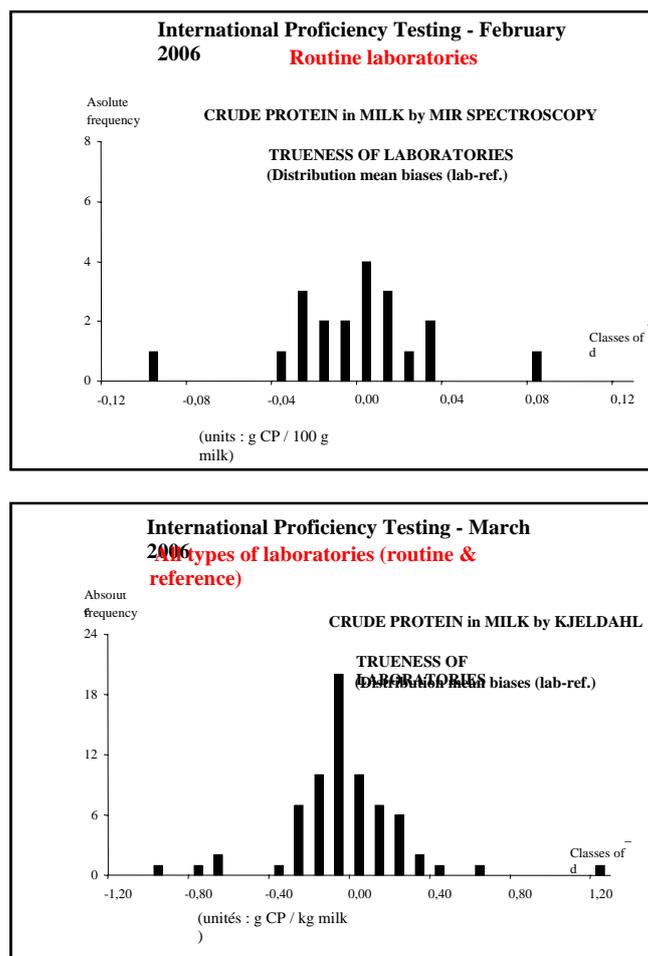


Figure 2. Example of comparison of laboratory bias distributions between mid infra red and reference protein determinations in recent international proficiency testing (Cecalait, 2006)

References

Anonymous. ISO 8196 | IDF 128 – Definition and evaluation of the overall accuracy of indirect methods of milk analysis – Application to calibration procedure and quality control in dairy laboratory.

Anonymous. IDF 141:2000 | ISO 9622:1999 - Whole milk -- Determination of milk fat, protein and lactose content -- Guidance on the operation of mid-infrared instruments.

Baumgartner c., Landgraf A., (2005): Deep frozen raw milk standards. The way from reference methods to reference systems. Proceedings of 34th Biennial Session of ICAR. Sousse, Tunisia, May 28-June 3, 2004. Performance Recording of Animals. State of the art, 2004. EAAP publication n°113, 2005, 253-257.

CECALAIT, 2006. Results of international proficiency testing in protein determination by mid infra red spectroscopy. February 2006. CECALAIT, BP 70129, F-39802 Poligny, France. www.cecalait.org.

CECALAIT, 2006. Results of international proficiency testing in protein determination by the Kjeldhal method. March 2006. CECALAIT, BP 70129, F-39802 Poligny, France. www.cecalait.org.

Grappin R., Lefier D., Pocher S., (1994): BCR Infra-Red Programme. Contract n°5554/1/5/374/91/11-BCR.F(10). Report of July 1994.

IDF Bulletin n°208, (1987): Monograph on rapid indirect methods for measurement of the major components of milk – A review of major advances and factors affecting the accuracy of the methods. www.fil-idf.org.

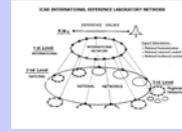
Leray, O., 1988; Protocole de préparation d'échantillons de lait reconstitués destinés à l'étalonnage des appareils infra-rouge. Note Technique ITEB-INRA Poligny n°1, France.

Leray, O., 1989; Ajustement/calcul des intercorrections des spectromètres utilisés pour les dosages TB-TP-TL du lait en moyen infra-rouge. Note Technique ITEB-INRA Poligny n°2, France.

Leray O., (1989): Influence de l'origine géographique du lait sur la précision des dosages de matière grasse et de protéines par spectroscopie dans le moyen infrarouge. *Le Lait*, 69, 547-560.

Leray, O., 1990; Procédure d'étalonnage des analyseurs infra-rouge au moyen de gammes d'échantillons de lait reconstitués. Proceedings of the 27th Biennial Session of ICAR. Paris.

Leray O., (1998): Quality control of conventional mid-infra-red milk analysers using recombined milk samples. Proceedings of 31st Biennial Session of the International Committee for Animal Recording (ICAR). Performance Recording of Animals. State of the art, 1998. Rotorua, New Zealand, 18-23 January, 1998. EAAP publication n°91, 1998, 131-138.



Reference and calibration system for routine milk testing

Advantages & Disadvantages - Choice

Olivier Leray, Cevalait, France

Meeting of ICAR Reference Laboratory Network, 6 June 2006 - ICAR Session Kuopio 2006 1

Introduction

Reference and calibration systems

⇒ refer to a **general analytical system** chosen for a prior defined purpose :
i.e. milk recording

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

Objectives

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

with providing **sufficient confidence** in the quality of results

and with an **acceptable balance** between **quality & cost**

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The error of measurement includes :**⇒ the precision error of the routine method :**

- repeatability & within day reproducibility (short term stability)
- under control
- cannot be avoided nor reduced in routine testing

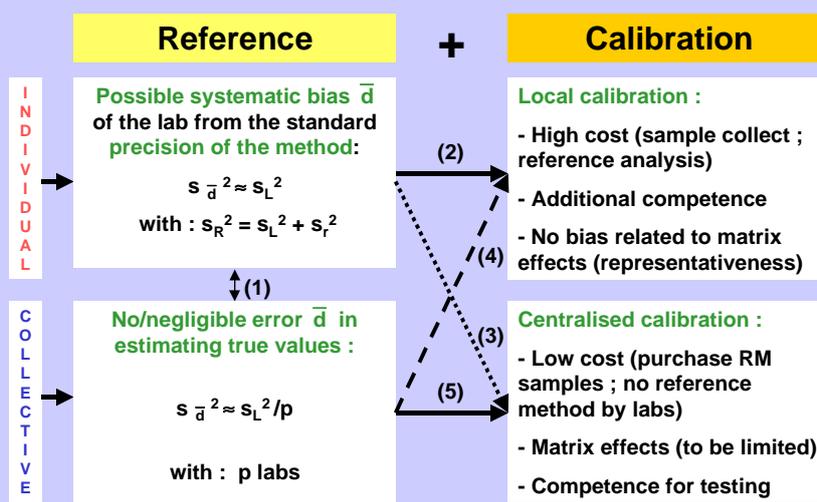
⇒ the precision error of the reference method : $S_R^2 = S_L^2 + S_r^2$

- **repeatability** : negligible as reduced by sample and replicate numbers
- **reproducibility** : Possible systematic error of the lab allowed by the method and normally distributed according to s_L

⇒ the error of calibration :

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

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Analytical systems available for milk recording

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

Mid infra red spectroscopy and matrix effects on classical wavelengths

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

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Elements for choice

Choice of appropriate methods

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

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

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

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

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Elements for choice

1°) Experimental evaluation

Over a one year period and on the whole region :

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

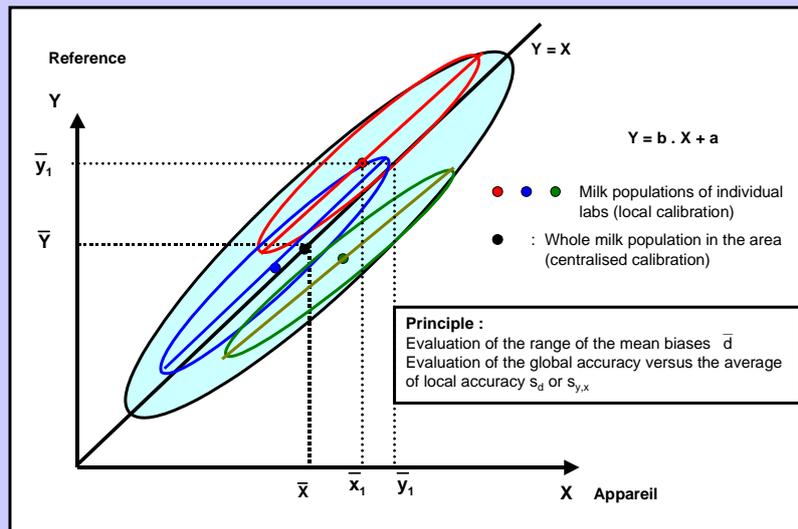
2- Evaluation : of ranges of variation of theoretical calibration bias between labs and between periods

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

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

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



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

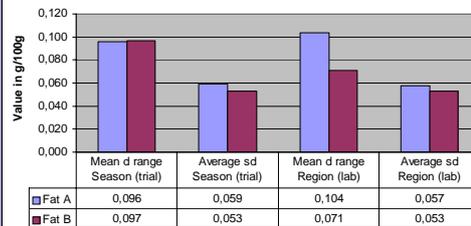
Samples of 8 regions of France with various geographical/seasonal situations analysed at the same time in reference and infra red and calibration (same instrument) optimised on the whole of data for each season to measure local effects (ANOVA).

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

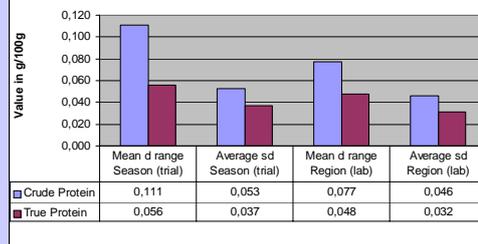
Units: g/100g

O. Leray, Le Lait, 69, 1989

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



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



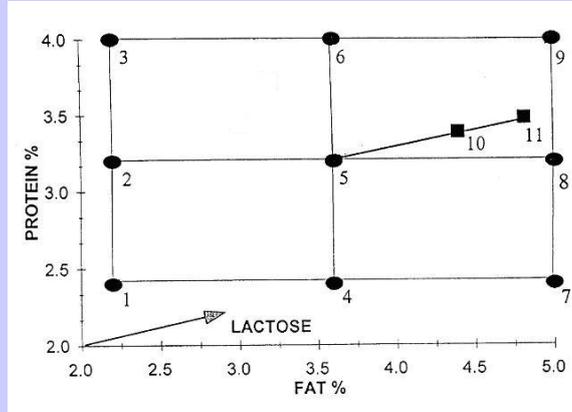
BCR MIR Prog. 1991:

15 European countries (labs)

8 trials on 1 year

2 bulk milks/trial/lab

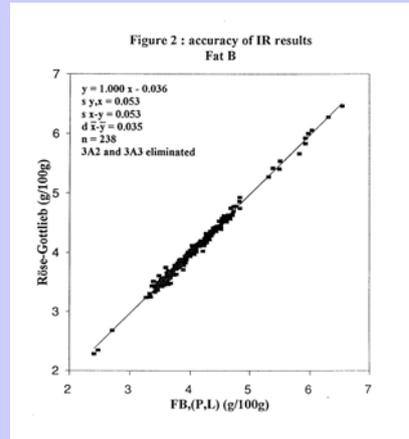
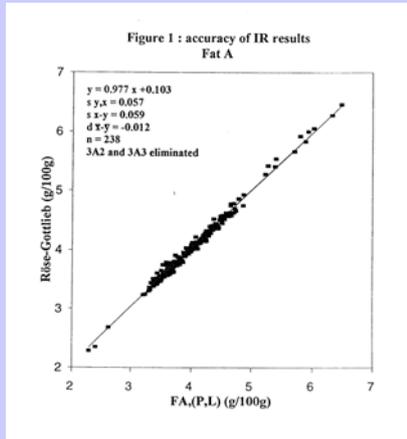
Appropriateness of recombined milk samples for centralised calibration



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

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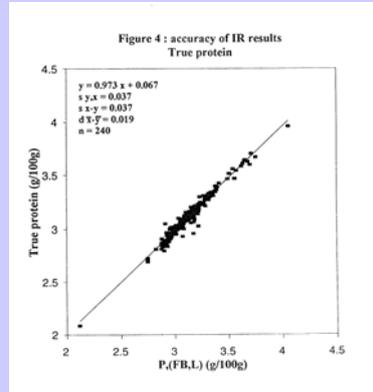
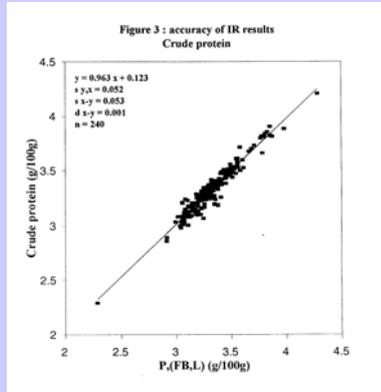
**Example : BCR MIR European Programme 1991
15 labs (countries) x 2 bulk milks x 8 periods of 1 year**



Calibration samples according to IDF 141

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**Example : BCR MIR European Programme 1991
15 labs (countries) x 2 bulk milks x 8 periods of 1 year**

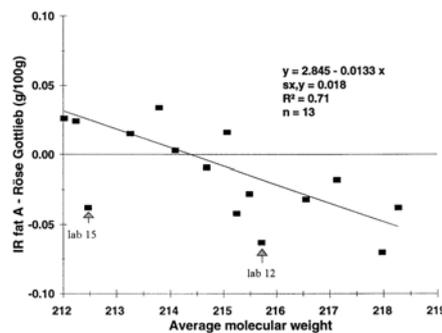


Calibration samples according to IDF 141

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**Matrix effects:
MW / Fat A**

Figure 9 : Influence of the variation between countries of the average fatty acids molecular weight of milk on the accuracy of IR fat A testing.

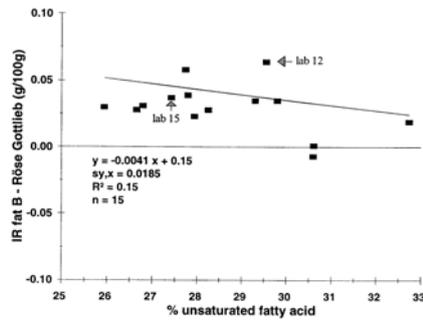


Each data point represent the mean of the analysis of 16 bulk milk samples collected over one year and analysed with a single instrument.
n = 15 European countries (laboratories); 2 laboratories discarded.

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**Matrix effects:
UFA / Fat B**

Figure 10 : Influence of the variation between countries of the proportion of unsaturated fatty acids of milk on the accuracy of IR fat B testing.

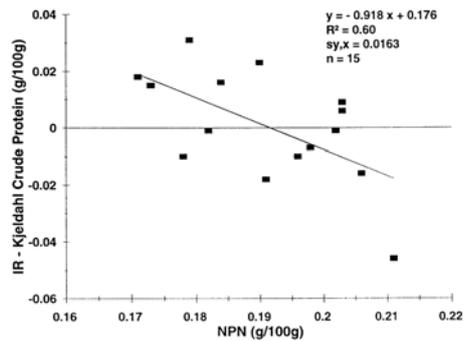


Each data point represent the mean of the analysis of 16 bulk milk samples collected over one year and analysed with a single instrument.
 $n = 15$ European countries (laboratories).

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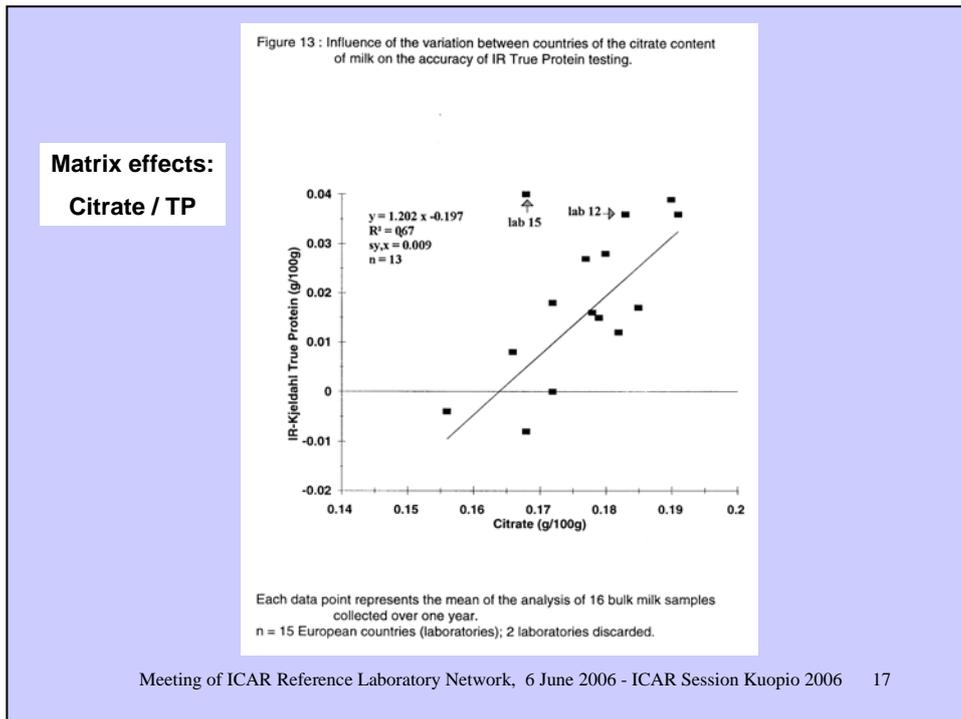
**Matrix effects:
NPN / CP**

Figure 12 : Influence of the variation between countries of the NPN content of milk on the accuracy of IR Crude Protein testing.



Each data point represents the mean of the analysis of 16 bulk milk samples collected over one year.
 $n = 15$ European countries (laboratories)

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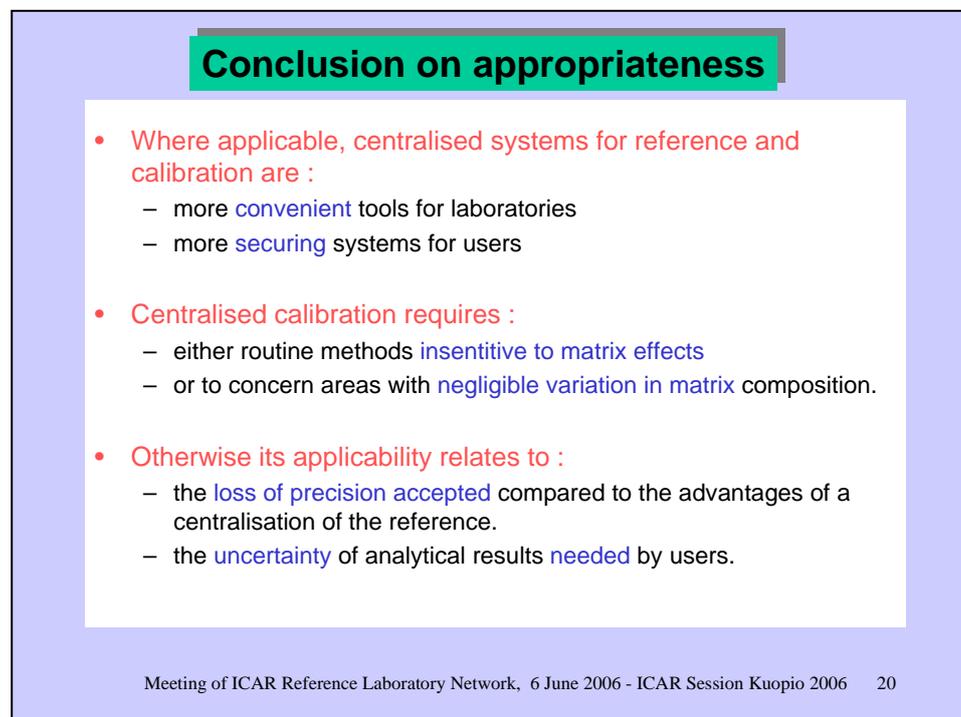
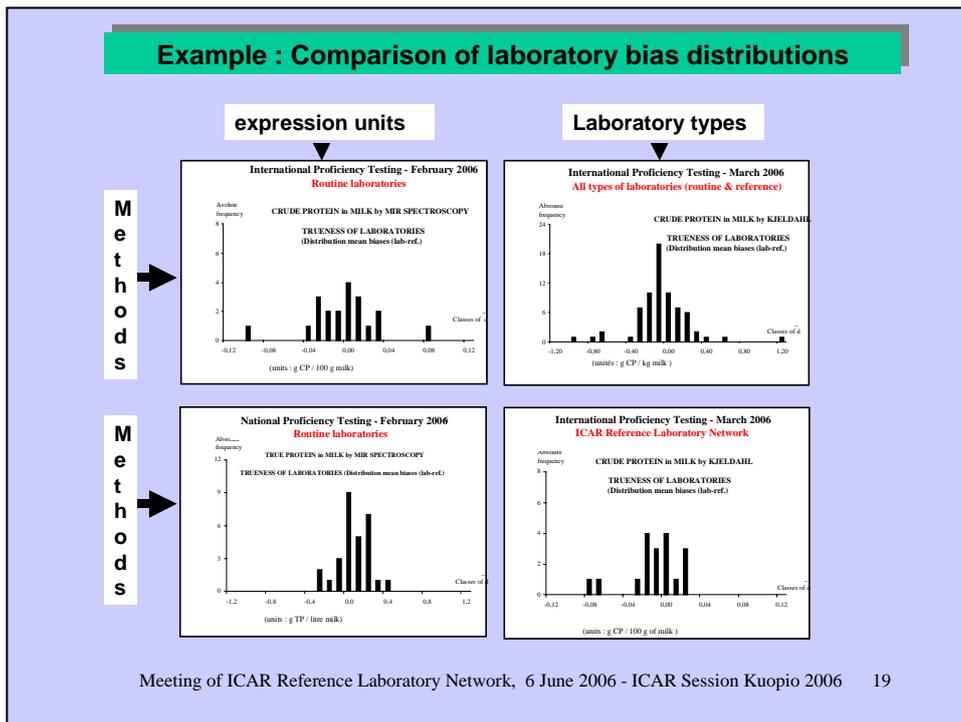


Elements of choice for a centralised calibration system

Comparison of of laboratory bias distributions in Proficiency Testing

- In centralised calibration :
laboratory bias (1) = bias with the reference method (2) + calibration bias (3)
 - MIR PTs : measures the distribution of laboratory biases (1)=(2)+(3)
 - PTs on reference methods : measures the distribution of biases with the reference method (2)
 - Comparing the standard deviations (or ranges) of biases between labs :
Routine method SD ≤ reference method SD ⇒ Improvement or equivalence (4)
Routine method SD > reference method SD ⇒ discrepancy of uncertainty (5)
- (4) => Centralised calibration system applicable.
- (5) => It is to users (milk recording) to consider whether or not the extent of discrepancy is acceptable for the intended use.

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Conclusion

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

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Thank You for your attention!

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National Reference System and Centralized Calibration in USA – Quality Certification Services, Quality Assurance Program, Unknown Sample Program

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Abstract

Quality Certification Services provides blind samples, “Sample Unknowns”, to all approved DHI laboratories on a monthly basis to conduct a centralized ring test. The samples are supplied by an approved reference laboratory and are tested by all DHI laboratories by the 2nd Friday of every month. The data is entered by each individual lab into an Internet web site and reviewed by the Lab Auditor. The results of the samples are supplied to the laboratories by the following Tuesday. This allows QCS to monitor all laboratories on a common set of samples.

Keywords: blind, sample unknowns, centralized, monitor

Introduction

QCS is a subsidiary of National DHI. QCS oversees 43 DHI laboratories and 4 DHI processing centers with enrollment of 4,200,000 cows. The Sample Unknowns Program is designed to help the genetic improvement of the dairy industry by showing the relationship of all laboratories when testing for fat, protein and SCC. The governing body of all genetic improvement is the Council for Dairy Cattle Breeding oversees all regulations for DHI testing.

How It Starts

Before achieving initial certification, laboratories must demonstrate acceptable machine performance by surpassing Sample Unknowns tolerances at least one time. Once that has been accomplished, the laboratory must submit to an on-site audit and demonstrate compliance with all aspects of the Code of Ethics and Uniform Data Collection Procedures manual.

Once certification has been established, laboratories will be subject to a biannual on-site audit in order to renew their certification. During the on-site audit, laboratories must allow the auditor to observe the routine analysis of samples. Laboratories failing to demonstrate routine compliance throughout the two-year period will become subject to annual on-site audits until consistent performance has been restored.

Monthly Audits of Sample Unknowns

Although the on-site audits are required for biannual laboratory certification Sample Unknowns must be submitted and found within acceptable limits on a monthly basis for ongoing certification to continue. This requirement must be met for each laboratory instrument used for the generation of sample results used in the *Genetic Evaluation Program* (GEP).

Auditing Guidelines of Infrared & SCC Instruments for Sample Unknowns

Each Laboratory receives refrigerated samples from the approved reference laboratory on the 2nd Tuesday of each month. The samples are comprised of 12 separate farms bulk tank milk. The samples are tested in duplicate. This ensures the instruments repeatability. The results are required to be submitted no later than 12 PM that Friday. (Figure 1) The auditor will review the results and post results the following Tuesday. The results are posted only for that DHI. (Figure 2)

Quality Certification Services, Inc.

Eastern Laboratory Services

Station 2 Foss 6500

Butterfat			Milk Urea Nitrogen			Protein			Somatic Cell Count		
1	0.00	13	0.00	1	0.00	13	0.00	1	0	13	0
2	0.00	14	0.00	2	0.00	14	0.00	2	0	14	0
3	0.00	15	0.00	3	0.00	15	0.00	3	0	15	0
4	0.00	16	0.00	4	0.00	16	0.00	4	0	16	0
5	0.00	17	0.00	5	0.00	17	0.00	5	0	17	0
6	0.00	18	0.00	6	0.00	18	0.00	6	0	18	0
7	0.00	19	0.00	7	0.00	19	0.00	7	0	19	0
8	0.00	20	0.00	8	0.00	20	0.00	8	0	20	0
9	0.00	21	0.00	9	0.00	21	0.00	9	0	21	0
10	0.00	22	0.00	10	0.00	22	0.00	10	0	22	0
11	0.00	23	0.00	11	0.00	23	0.00	11	0	23	0
12	0.00	24	0.00	12	0.00	24	0.00	12	0	24	0
Tot											

Figure 1. Unknowns Data Entry Web Form.

Protein													
Sample Number	Lab/Ref	Instrument	Avg	Instr Diff	Results	Rep1	Rep2	Prec Range	Stats SD	Reps	Accuracy IR	Mean	Diff
1	3.030	3.033	0.003		3.04	3.04	0.000	0.000		3.040	0.010		
2	3.183	3.169	-0.014		3.14	3.16	0.020	0.014		3.150	-0.033		
3	3.080	3.057	-0.023		3.06	3.06	0.000	0.000		3.060	-0.020		
4	3.047	3.036	-0.011		3.02	3.03	0.010	0.007		3.025	-0.022		
5	3.127	3.114	-0.013		3.12	3.13	0.010	0.007		3.125	-0.002		
6	3.210	3.206	-0.004		3.19	3.21	0.020	0.014		3.200	-0.010		
7	2.993	2.999	0.006		2.98	3.00	0.020	0.014		2.990	-0.003		
8	3.490	3.464	-0.026		3.45	3.46	0.010	0.007		3.455	-0.035		
9	3.340	3.322	-0.018		3.31	3.32	0.010	0.007		3.315	-0.025		
10	3.350	3.349	-0.001		3.35	3.35	0.000	0.000		3.350	0.000		
11	3.070	3.053	-0.017		3.04	3.05	0.010	0.007		3.045	-0.025		
12	3.033	3.028	-0.005		3.03	3.03	0.000	0.000		3.030	-0.003		
		MD -0.010				SDA 0.006				MD -0.014			
		SDD 0.010								SDD 0.015			

Figure 2. Unknown Sample Report For Infrared Protein Test.

Acceptable Readings for Calibration Checks for Infrared

The mean difference must not exceed 0.05% and the standard deviation of differences must not exceed 0.06% in three of the previous four trials.

The rolling mean difference over six trials may not exceed .02% (Figure 3)

Changes being considered in 2007 for tighter tolerances

Acceptable Readings for Calibration Checks for Electronic SCC

The mean percent difference must not exceed 10%. And the SD must not exceed 10% in three of the previous four trials. The rolling mean difference over six trials may not exceed 5%

****Changes being considered in 2007 for tighter tolerances****

Machine History						
Month	FAT Results			PRO Results		
	MD	SDD	RMD	MD	SDD	RMD
Dec	-0.034	0.015	-0.034	-0.030	0.013	-0.030
Jan	0.024	0.036	-0.005	0.013	0.021	-0.009
Feb	-0.051	0.051	-0.020	-0.017	0.014	-0.011

Figure 3. Machine History for Fat & Protein

Quality Certification Services

Quality Assurance Program Sample Unknowns

Jere High
USA

Lab Manager & Technology Services
Lancaster Dairy Herd Improvement Association
WWW.LANCASTERDhia.COM

President
North American Lab Managers Association
WWW.NALMA.ORG

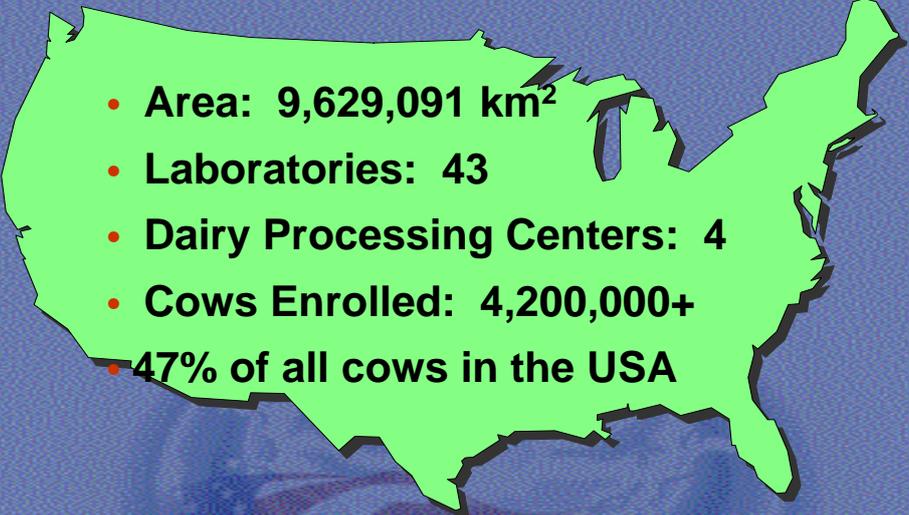
Council for Dairy Cattle Breeding

National Dairy Herd Improvement Association

Quality Certification Services



United States of America

- 
- Area: 9,629,091 km²
 - Laboratories: 43
 - Dairy Processing Centers: 4
 - Cows Enrolled: 4,200,000+
 - 47% of all cows in the USA

Laboratory Certification and Audits

of

Sample Unknowns

Initial Certification Audits

- Before achieving initial certification, laboratories must demonstrate acceptable machine performance by surpassing Sample Unknowns tolerances at least one time. Once that has been accomplished, the laboratory must submit to an on-site audit and demonstrate compliance with all aspects of this manual and with the *Code of Ethics* and *Uniform Data Collection Procedures*.

Initial Certification Audits

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Monthly Audits of the Sample Unknowns

- Although the on-site audits are required for biannual laboratory certification, Sample Unknowns must be submitted and found within acceptable limits on a monthly basis for ongoing certification to continue. This requirement must be met for each laboratory machine used for the generation of sample results used in the *GEP*.

Auditing of Infrared and SCC Instruments for Sample Unknowns

Calibration Check Procedure

- On a monthly basis, the laboratory must purchase duplicate sets of 12 samples from a supplier designated by the auditor. The samples must be analyzed and the following data submitted to a predetermined site by midnight EST on the second Friday of each month.

Acceptable Readings for Calibration Checks IR

- The mean difference must not exceed 0.05% and the standard deviation of differences must not exceed 0.06% in three of the previous four trials.
- The rolling mean difference over six trials may not exceed .02%
- ** Changes being done in 2007 for tighter tolerances **

Acceptable Readings for Electronic SCC

- The mean percent difference must not exceed 10%. And the SD must not exceed 10% in three of the previous four trials.
- The rolling mean difference over six trials may not exceed 5%

“Unknown Sample” Web site



Quality Certification Services
 Suite #102 - 3021 E. Dublin Granville Rd.
 Columbus, OH 43231
 Phone: 614-890-3630 - Fax: 614-890-3667
 E-mail: dhia@dhia.org

Welcome To Quality Certification Services, Inc.

Please Enter Your User ID and Password

User ID:

Password:

Sample Number	Lab/Instrument			Avg Diff	Protein Results		Prec Stats		Accuracy Stats		
	Ref	Inst	Diff		Rep1	Rep2	Range	SD	Reps	IR Mean	Diff
1	3.030	3.033	0.003	3.04	3.04	0.000	0.000	3.040	0.010		
2	3.183	3.169	-0.014	3.14	3.16	0.020	0.014	3.150	-0.033		
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8	3.490	3.464	-0.026	3.45	3.46	0.010	0.007	3.455	-0.035		
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12	3.033	3.028	-0.005	3.03	3.03	0.000	0.000	3.030	-0.003		
MD -0.010				SDA 0.006				MD -0.014			
SDD 0.010								SDD 0.015			

LANDHIA
B2500 B

Machine History

Month	FAT Results			PRO Results		
	MD	SDD	RMD	MD	SDD	RMD
Dec	-0.034	0.015	-0.034	-0.030	0.013	-0.030
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Feb	-0.051	0.051	-0.020	-0.017	0.014	-0.011

Thank you
&
Questions?

What is the required Accuracy of a Test related to Genetic Improvement

Hans Wilmink

NRS, PO Box 454, 6800 AL Arnhem (NL)

E mail: Wilmink.H@nrs.nl

In 1998 a report is presented by an ICAR-INTERBULL task force group to the ICAR board on the effect of accuracy of recording on genetic progress. Member of the task force group were: Petra Galesloot (NL), Dave Johnson (NZ), Wijbrand Ouweltjes (NL), Andrea Rosati (IT), Larry Schaeffer (CAN), Torstein Steine (N), Paul VanRaden (USA), Hans Wilmink (NL, convenor). The main conclusion in that report were that genetic gain is hardly effected if recording intervals increase to 8 weeks; that test in mid lactations have most predictive value for 305 day records; that a decrease meter accuracy with 1 kg is equivalent to increase of recording interval with 1 week; that is more important to invest in data quality check by storage of recorded data in an integrated database. The recommendations to the ICAR board were therefore that meter accuracy can be relaxed; that minimum number of tests in lactation should be 4 and that recording systems should be more flexible.

Galesloot and Ouweltjes (1998) studied the effect of different rates of accuracy of a milk meter on the heritability a measured trait and thus on genetic progress. In case of daily measurements, no decrease in heritability was could if meters became less accurate till 10%. In case of recording at 4 weekly intervals, the decrease in heritability was 0.02 if meters become 10% less accurate. In case of recording at 8 weekly intervals this decrease was also 0.02.

It is concluded that the accuracy of meters can be relaxed, at least till 10%. This will not effect genetic progress. It is still important that meters are unbiased. Main emphasis should be to keep the costs for recording al low as possible in order to make recording attractive in the market. Thus a significant reduction of costs of meters or costs for analysis of the milk content would be most important. In line measurements of milk content would be still of interest, if accuracy is low, but bias is zero.

What is the required Accuracy of a Test related to Genetic Improvement



Dr. Hans Wilmink,
June 2006

NRS is een onderdeel van CRV Holding BV

Report of ICAR/ INTERBULL working group

- Report to ICAR board, 1998
- Objectives:
 - ▶ Effect of test interval (with and without measurement error) on accuracy of lactation;
 - ▶ Effect of accuracy of lactation on genetic progress;
 - ▶ Recommendations to ICAR.



Conclusions

- Milk yield: decrease accuracy meter with 1 kg is equivalent with increase test interval with 1 week
- Till 8 weekly recording, genetic gain is hardly effected
- Tests in mid lactations are best
- Need for reliability measures in 305 day records
- Improve methods to calculate 305 day yields



Conclusions

- More animals in recording better than more tests per animal
- Invest in data quality check:
 - ▶ Use of integrated Recording Registration System
 - ▶ Most genetic loss by faulty data
 - ▶ develop statistics to access accuracy and report to farmers



Recommendations to ICAR

- Be flexible in Recording schemes: offer more options
- Frequency from all milkings to 4-5 tests per 305 days
- ICAR Accuracy of meters can be relaxed (till 7.5%?)
- A lactation record must be transparent (showing accuracy of record)



Effect of measurement error on heritability



NRS is een onderdeel van CRV Holding BV

Study Galesloot, Ouweltjes, Wilmink (1998)

- Dataset of 4870 lactations with all milking records
- Simulation of the inaccuracy of meter



Heritability at different rates of Accuracy meter

Number of tests	Weekly interval	Hertability 305 day milk		
		0,05%	5%	10%
	daily	0,30	0,30	0,30
43	1	0,30	0,30	0,29
22	2	0,29	0,29	0,29
15	3	0,29	0,29	0,28
10	4	0,29	0,28	0,27
9	5	0,28	0,28	0,27
8	6	0,28	0,28	0,26
7	7	0,28	0,27	0,25
6	8	0,27	0,27	0,25
5	9	0,27	0,26	0,24



Discussion

- Accuracy of meters must be relaxed (till 10%)
- Meters must be unbiased
- Invest in:
 - ▶ cheap unbiased meters so that data collection process can be more automated
 - ▶ reduction of costs for analysis of milk content (allow lower accuracy)



Discussion and conclusion

Notes by : Gavin Scott, SAITL, Hamilton, New Zealand

Part 1

1. *ICAR AQA strategy and prospective for the network*, Olivier Leray (Fr)
2. *Policies of ICAR about Milk Analysis*, Andrea Rosati (IT)

Discussion:

Question: *Did board discuss whether QA should be user-pays or part of the normal ICAR fee?*

Answer: -Nations have different expectations (eg. Dutch vs German vs developing countries).
-QA is a tool that is most important to help developing countries to improve their systems.
-Payment is only to cover costs – ie. For an auditor to go to Romania to spend two days would have two days of travel plus 1 day writing report therefore 5 days in total (therefore big cost).
-ICAR philosophy is to raise all countries to the same level.

Question: *As a laboratory that is accredited to ISO 17025 what does ICAR certification bring above 17025? (ie. Do you need if you have 17025?)*

Answer: -Situation is different – ICAR is recording benchmark.
-ICAR could take into consideration that a lab is accredited to 17025 – therefore certification process easier / shorter.

Questions: *Is there a possibility to harmonise (integrated approach) so no overlap with other organisations (EU food standards...)?*

Answer: -ICAR has own specificities.
-Not going into empty space.
-There are many areas not covered, particularly for international level.
-However need to keep an eye out to see what other organisation are doing. For example the DNA labs are using an existing DNA ringlab rather than developing its own.

Remark: ICAR = total process from farm to lab to farmer...

Question: *Regarding intro of QA certification – What happens to labs that are currently using the special stamp?*

Answer: Special stamp kept for next 3 years, then start QA cert process. May not need initial visit.

Question: *What about using stamp on documents?*

Answer: Needs to be discussed by ICAR (board or General assembly??) – probably keep special stamp, or have extra special stamp.

Question: *Is process going to be on a sliding scale or pass/fail?*

Answer: Sliding scale

Presentations:

- | | |
|--|-----------------------|
| 1. <i>Reference system - Principle and practice,</i>
(DE) | Christian Baumgartner |
| 2. <i>Reference and calibration systems for routine milk testing: Advantages / disadvantages, choice criteria,</i> | Olivier Leray (FR) |
| 3. <i>Example of national reference system and centralised calibration,</i> | Jere High (USA) |
| 4. <i>What would be the required accuracy of a milk test and its components with view on genetic progress,</i> | Hans Wilmink (NL) |

Discussion:

Question: *You discussed impact of variation (accuracy) on Genetic Improvement, what is the impact from the herd management perspective?*

Answer: -Study was related to effects on genetic improvement
-Daily management changes day to day Thus in that case you would not want to have variation. In that case you may use week averages to monitor management, but day to day would be better.

Question: *You showed impact of accuracy of the meter on breeding value. Would there be any reshuffling of animals within the herd by decreased accuracy?*

Answer: Only a little bit

Question: *You indicated that accuracy not as important as not being biased. Is it possible to be less accurate but not biased?*

Answer: -If can't be unbiased without being precise then you still have accurate meters and this presentation is not so relevant
-At the end of day need you want to get costs down. This is most important: reducing costs for recording. If that can with less accurate meters, but still unbiased, this is OK.

Comment 1: -Accuracy vs price. Main part of price of test equipment relate to other things not necessarily related to making equipment accurate.

Comment 2: -Another approach if bias is key is to throw limits away
-How to test full system milkmeter – ID – sample

Comment 3: -Must be transparent (sources of error) right through system.

Question: *Relating to Competent authorities – are ICAR looking at playing a part to link to other side?*

Answer 1: -ICAR has contacts with other authorities (eg EU legislation)
-However ICAR links worldwide so not easy

Answer 2: -ICAR could manage SCC system worldwide
-EU only sets limit on test
-ICAR could take lead indirectly and impact the other side by doing so.

Answer 3: -EU has contact to help develop guidelines

-They are not expert in these fields so look to the experts for direction.

Question: *Will you supply protocol for national approval?*

Answer: Yes – evaluation by countries first - harmonised evaluation.

Question: *Connection between USA vs ICAR ring test?*

Answer If standard agreed then could work

Question: *In the USA Proficiency program what matrix is used (ie. Preserved, raw etc.)*

Answer: -Wholemilk, not preserved.
-Same set for component and SCC.
-Separate set for Direct microscopic SCC method. Jere indicated that they also put this set through the autoanalysers.

Question: *Is there just one supplier of the proficiency program?*

Answer: -Only one supplier in the USA for the Proficiency program
-many suppliers for the calibration samples – labs choose.

Question: *Is the results from the instrument trial compared against the reference method or an average of the instrument results summated?*

Answer: Compared against a reference average established using 7 labs.

Conclusion of the meeting

The meeting has been the occasion to ICAR to present the Quality Certification system under implementation and re-situate the part devoted to milk analysis and laboratories in the whole system. Analytical issues are of key importance for dairy genetic and farm management, which is stressed again, and comparability, equivalence and confidence are the targets for a harmonised AQA system to be strengthened further.

A variety of possible future actions to be undertaken by the working group MTL WG have been listed. They should be hierachised and developed by the group under its next new status of Sub-Committee.

The Chairman thanked the attendance for their large participation, invited every attending person to take part in the meeting of MTL WG to be held from 2.00 p.m. and, for those leaving, addressed his best greetings, taking date for a next "Rendez-vous" in 2008.