"International Strategies and New Developments in Milk Analysis"

Sixth ICAR
Reference Laboratory Network Meeting

28th May 2012

38th ICAR Session, Cork, Ireland

MASC
ICAR Sub-Committee
on Milk Analysis

FOREWORD

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

Year 2006 marked the end of the first 10-year period of the implementation/development of the AQA system of ICAR. From Kuopio, it was decided to produce practical guidances and tools to facilitate the work of reference and routine laboratories in ICAR countries through harmonised practices, and establish a reference frame to obtain the ICAR Certificate of Quality.

That decision has directed the programme of the following conference sessions. From 2010 it involves a core part specifically dedicated to AQA, lab network and reference system issues and a second part to focus on new topics and development in analytical methods.

In June 2008 in Niagara Falls (USA) focus was made on the international traceability, anchorage, reference systems, centralized calibration and analytical equivalence. In June 2010 in Riga (Latvia), the interest was given to the measurement of ICAR AQA system efficiency, developments in reference systems and the latest outcomes in milk analysis including mid infrared and on-farm milk analysis. In 2012, in the continuation of Niagara Falls and Riga, the conference session will update on the latest progress in the reference system developments and new issues and challenges in mid infrared milk analysis.

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

Poligny, 31st August 2012

On behalf of ICAR Sub-Committee on Milk Analysis

Olivier Leray
Chairman of the organizing committee

Programme committee:	ICAR Sub-Committee on Milk Analysis
Organizing committee:	Christian Baumgartner Harrie van den Bijgaart Olivier Leray Silvia Orlandini
Session chairperson:	Olivier Leray

International Strategies and New Developments In Milk Analysis

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List of participants

Last name	First name	Organisation	Country
Asmussen	Tove	Raw Milk Connect	Denmark
Asmussen	Berte	Raw Milk Connect	Denmark
Baumgartner	Christian	Milchprüfring Bayern e.V.	Germany
Broutin	Pierre	Bentley Instruments Europe	France
Burke	Martin	Irish Cattle Breeding Federation - ICBF	Ireland
Cassandro	Martino	Padova University	Italy
Chang	Chu-li	Hsin-chu Branch, Livestock Institute	Taiwan
Colinet	Frédéric	Gembloux Agro Bio-Tech - ULG	Belgium
Craven	Tony	National Milk Recording	United Kingdom
De Marchi	Massimo	Padova University	Italy
Dilan	Sahin	Cattle Breeder's Organization of Turkey	Turkey
Fait	Gianfranco	Foss Italy S.p.A.	Italy
Fernandez	Juan	CRA-W Gembloux	Belgium
Ferrand	Marion	Institut de l'Elevage	France
Goubau	Amaury	Gembloux Agro Bio-Tech - ULG	Belgium
Grelet	Clément	CRA-W Gembloux	Belgium
Hammami	Hedi	Gembloux Agro Bio-Tech, - ULG	Belgium
Hanus	Oto	Research Institute for Cattle Breeding	Czech Republic
Kold-Christensen	Steen	Foss Analytical A/S	Denmark
Krencik	Dorota	National Animal Breeding Centre	Poland
Lemmer	Friedhelm	Lemmer-FullWood GmbH	Germany

Last name	First name	Organisation	Country
Leray	Olivier	FGE-ICAR	France
Lombard	Rian	Lactolab	South Africa
Lynch	Mike	Irish Cattle Breeding Federation - ICBF	Ireland
Miggliore	Filippo	AAFC – Guelph University	Canada
Mulawa	Katarzyna	Foss Analytical A/S	Denmark
Orlandini	Silvia	Laboratorio Standard Latte – AIA	Italy
Potočnik	Klemen	Ljubljana University, Biotechnical Faculty	Slovenia
Rhoads	John	Eastern Laboratory Services	United States
Rossoni	Attilio	Italian Brown Cattle Breders Association	Italy
Sang-Min	Lim	Milk Component Analysis Centre - AIAK	Korea
Savickis	Salius	State Laboratory Pieno Tyrimai	Lithuania
Scott	Gavin	MilkTest-NZ	New Zealand
Sievert	Steven	National DHIA	United States
Van den Bijgaart	Harrie	Qlip - Zutphen	The Neterhlands
Velioğlu	Hüseyin	Cattle Breeder's Organization of Turkey	Turkey
Werner	Andreas	LKV BW e.V.	Germany

Opening and introduction

Olivier Leray (Session Chair)

France Génétique Elevage-ICAR, 149 rue de Bercy, F-75595 Paris cedex 12, France

The Chairman welcomed the participants and introduced the meeting. He reminded the conference meeting is not an ordinary technical session since at the origin it was thought and organised for the members of ICAR Reference Laboratory Network so as to enable them to benefit from regular contact with their peers and ICAR experts. Information exchange is expected to allow a harmonious evolution of the ICAR AQA system through good understanding and consensus. Nevertheless the largest attendance which is any interested person in dairy analytics is welcome as this enable the SubCommittee on Milk Analysis to disseminate broadly the most relevant information on ICAR analytical strategies and thereby promote the ICAR AQA system.

He especially mentioned that such conference meetings constitute open forums for all analytical people and invited attendees not to hesitate to enter discussion and ask questions. He informed that any suggestions and proposals made during that meeting can serve for a brainstorming analytical workshop scheduled the next day in the streamline of the definition of new analytical strategies to propose to the ICAR Board.

He presented the programme formerly announced and split in two parts and launched the first presentation series.

International Strategies and New Developments

In Milk Analysis

Part 1: Reference systems – New developments

"International Strategies and New Developments in Milk Analysis"

14.00:   Opening – Welcome & introduction

Part 1:   **Reference systems – New developments**

14.15-14.35:   **ICAR Reference Laboratory Network – Objectives & stage of progress,**
                                                                    Olivier Leray (FGE-ICAR, FR)

14.35-15.00:   **International survey on somatic cell counting – Situation of lab network**
                    organisation and practices,   *Silvia Orlandini (LSL-AIA, IT)*

15.00-15.25:   **PT scheme interlinkage and international laboratory anchorage,**
                                                                    Olivier Leray (FGE-ICAR, FR)

15.25-15.50:   **Presentation on Irish milk recording analysis and reference laboratory in the**
                    ICAR analytical reference system,   *Martin Burke (ICBF, IE)*

15.50-16.15:   **Discussion**

16.15-16.45:   **Coffee break**

ICAR Reference Laboratory Network - Objectives & Stage of Progress in 2012

Olivier Leray

France Génétique Elevage-ICAR, 149 rue de Bercy, F-75595 Paris cedex 12, France

Abstract

With the important development of dairy genetic trade worldwide and international genetic evaluation, assuring quality and comparability of measurements and recordings between countries has become of an utmost importance as the bases of fair and loyal commercial exchanges and the support for suitable confidence in genetic values estimation.

Since 1994 ICAR has been developing an analytical quality assurance system (AQA) in order to harmonise analytical data quality within ICAR. An international network, the so-called reference laboratory network, was created in 1996 as a platform to relay good laboratory practices, guidelines and standards and support routine milk testing laboratories with services.

ICAR has implemented a regular annual programme in order to provide network members with traceability and anchorage to an international reference. Complying with the ICAR AQA system has become a requirement to obtain the ICAR Certificate of Quality for milk analysis.

Progress of the ICAR Reference Laboratory Network development is regularly measured at the occasion of ICAR biennial sessions and orientation discussed for further beneficial developments. Extending the network membership to new member organisations is a major objective together with enhancing participation in the international proficiency study programme managed by ICAR.

Keywords: milk analysis, reference laboratories, network, proficiency testing, analytical harmonisation

Introduction

For the two last decades, with the worldwide economic globalization, the international dairy genetic trade have increased dramatically while the genetic evaluation has evolved towards the international scale with processing data obtained in numerous different countries (re Interbul). In such a context comparable measurements and recordings are the bases of equivalent estimation of animal genetic values between countries and fair and loyal commercial exchanges. In order to support milk analysis and assure a harmonised quality of analytical data within ICAR, an analytical quality assurance system (AQA) was developed by ICAR from 1996 and an international network of so-called reference laboratory network was created as a platform to relay good laboratory practices, guidelines and standard and support routine milk testing laboratories with services. The AQA system managed and developed by the ICAR Sub-Committee on Milk Analysis (MASC) has taken place in a more general quality certification implemented by ICAR from 2006 in genetic recording activities. In order to obtain the ICAR Quality Certificate the applicant member organisations should meet the recommendations stated in ICAR guidelines with respect to milk analysis and analytical quality control, bring proof of regular participation in proficiency testing schemes and demonstrate precision traceability through an international anchorage.

To allow such an anchorage since 1996 ICAR has organised annual proficiency study programme on milk analysis methods used in milk recording (i.e. fat, protein, lactose, urea, somatic cell counting) twice a year using Actilait-Cecalait services (France). The quality control objectives and possible use of results for traceability and international anchorage were describes in former ICAR session in 2008 and 2010.

This is the occasion at ICAR biennial session to present the development progress of the ICAR Reference Laboratory Network , as already made in Niagara falls 2008 and Riga 2010, and to discuss on orientations and further beneficial development and orientation.

Evolution of network membership

Since it was created in 1996, ICAR reference laboratory network has progressively grown up to reach stabilisation in 2003. Then the membership was maintained stable - but a little decrease in 2005 and 2006 before recovery at 38 members - until 2010 where the network welcomed three new members:

- the Reference Laboratory for Milk and Dairy Science (Zagreb, Croatia),
- the Japan Dairy Technical Association (Tokyo, Japan)
- the Deutscher Verband für Leistungs und Qualitätsprüfungen e.V. (Bonn, Germany).

At the end of 2010, the network is composed of 41 laboratory members from 34 countries according to Table 1.

Table 1. Country composition of ICAR reference Laboratory Network (n : number of member(s))

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

By referring to 52 countries of the whole of ICAR member organisations, the proportion of represented countries is 65 p. cent and although not the ICAR member organisations do not deal all with milk production there is still place for welcoming new member laboratories in the future according to the evolution of milk analysis organisation in those not yet represented countries. The Sub-Committee on Milk Analysis identified three areas where a larger representation is needed : Latin America, Asia and CIS, while in the others (Europe, Africa, Oceania) complementation would be needed (see figure 2).
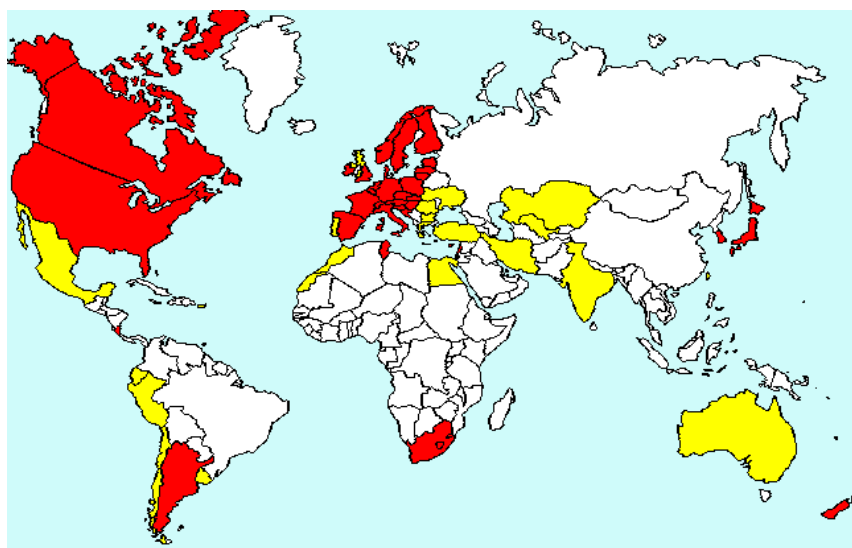

Figure 1. Distribution of member countries (Red = Network member countries; Yellow = ICAR member countries)

Eligibility criteria and evolution of competence within the network

A laboratory acquires the status of member of ICAR Reference Laboratory Network through its nomination by an ICAR member organisation of its country. The status of so-called reference laboratory

implies as prerequisites well-defined competences and roles for the sake of milk analysis and milk testing laboratories of its country. They are the followings:

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

Exception to the rule may be made for countries that have but few routine laboratories, with no way to join a neighbour country system therefore need to link directly to the international level.

From the network launching the number of eligibility criteria met by laboratories has accompanied the membership increase (Figures 2 and 3) but for different levels. The most represented roles are ring test organizers, reference materials suppliers, information in analytical matter and training in laboratory techniques whereas evaluation of analytical methods / instruments, research in analytical methods and national regulatory control are represented in lower proportions in the network.
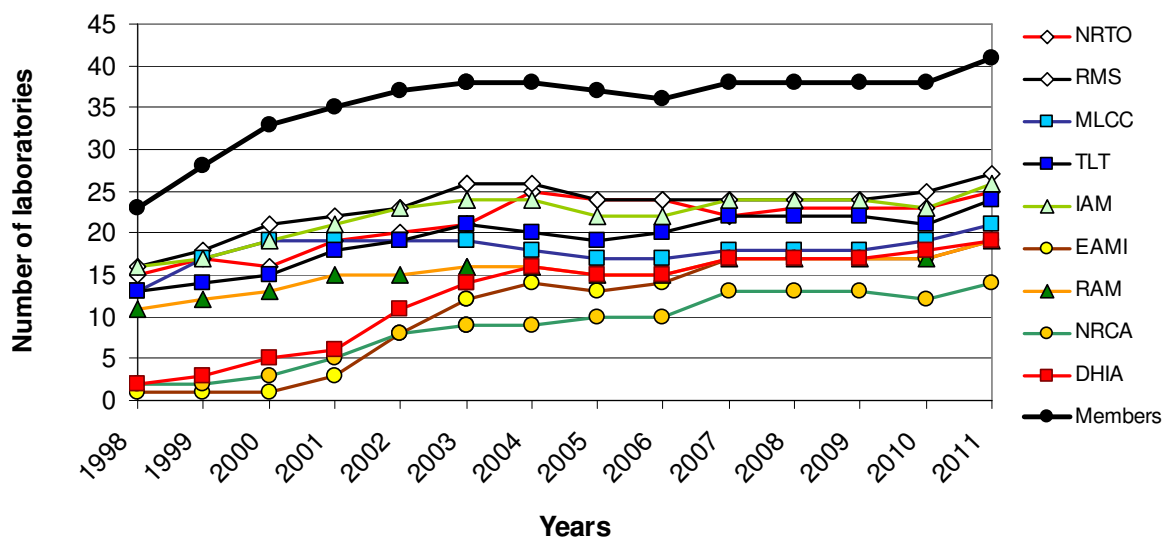

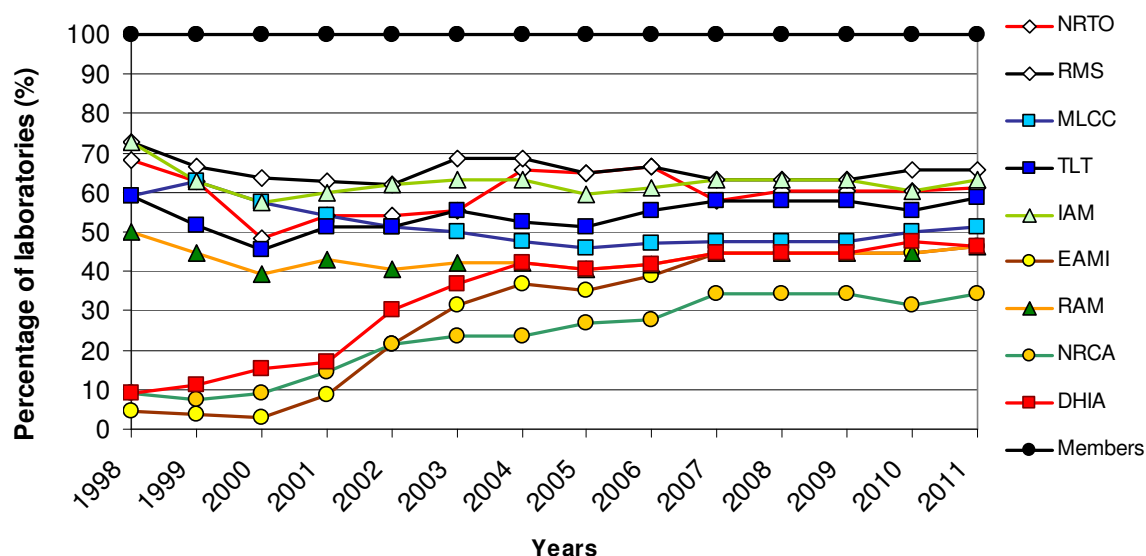Figure 2. Evolution of membership and missions - Numbers

Figure 3. Evolution of membership and missions – Percentages

The degree of qualification of the network is marked by the proportion of laboratories showing the largest number of eligibility criteria. The highest the proportion the greater the efficiency in playing the expected role for ICAR. By referring to Table 2, in 2011 more than 17 p. cent of labs show all the criteria requested, 40 p. cent at least 6 (75% of criteria) and 63 p. cent at least 4 (50% of criteria). 6 of laboratories (15%) are isolated milk testing laboratories directly anchored to the ICAR ref lab network.

Table 2. Numbers and proportions of eligibility criteria of network members in 2011.

Criteria		with N		with at least N	
N	%	Lab number	Lab %	Lab number	Lab %
8	100%	7	17%	7	17%
7	88%	5	12%	12	29%
6	75%	4	10%	16	39%
5	63%	3	7%	19	46%
4	50%	7	17%	26	63%
3	38%	3	7%	29	71%
2	25%	2	5%	31	76%
1	13%	4	10%	35	85%
0	0%	6	15%	41	100%

Evolution of participation in annual proficiency study programmes

Participation in international ICAR PT trial has slightly reduced from 2009 in fat and protein and the level appears stabilized around 16 participants per trial. For lactose participation has been more fluctuant as moving back and forth between about 10 and 20 participants per trial. From 2010 this is about 15 labs participating. In somatic cell counting a similar trend can be noted - although from less time as trials started only in 1999 - with a last position at 15 participants, whereas participation in urea is relatively regular around 14.

In general the numbers of participant in trials on milk component that are used in genetic evaluation schemes (i.e. fat, protein, SCC) appears regularly about or slightly less than the half of the

network membership number. This is the indication of that the implementation of the ICAR AQA system is not yet completed and still on-going within ICAR. The underway process should be continuously explained, promoted and come to concrete outcomes through practical analytical services to laboratories and member organisations.
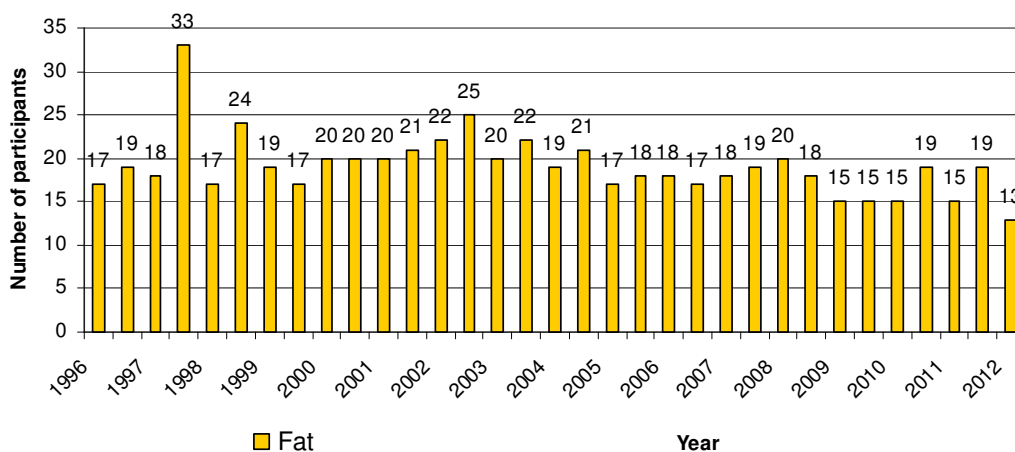

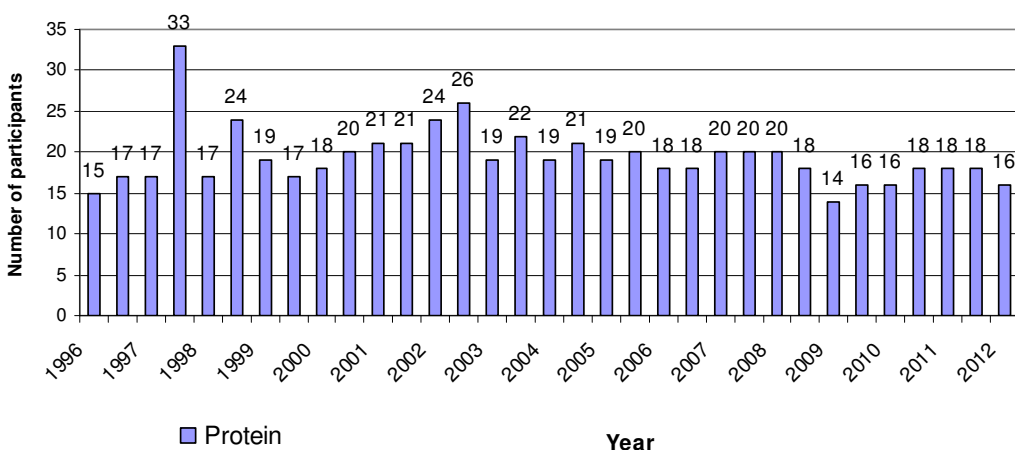Figure 4. Evolution of participation in ICAR PTs for Fat.

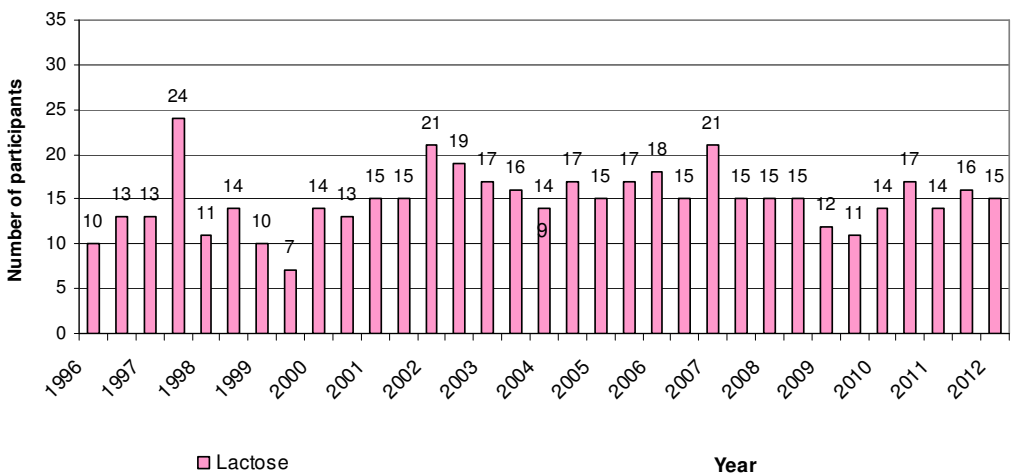Figure 5. Evolution of participation in ICAR PTs for Protein.

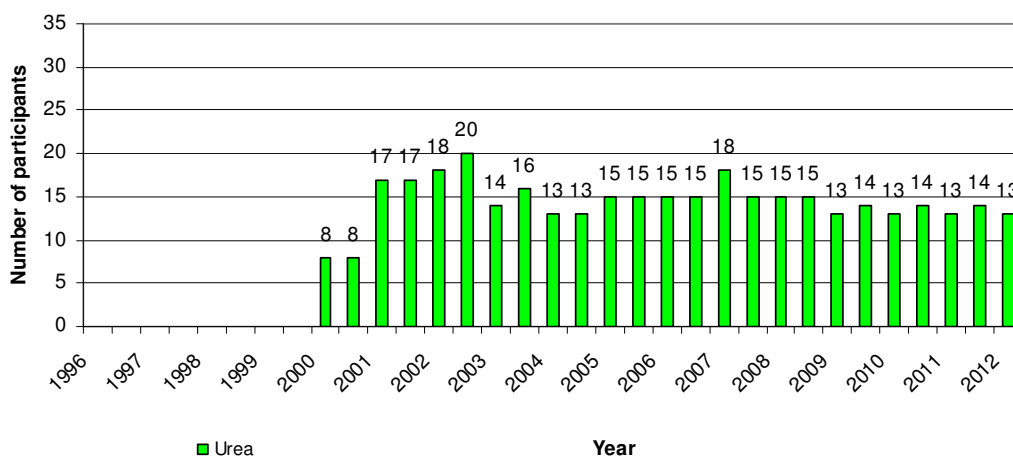Figure 6. Evolution of participation in ICAR PTs for lactose.

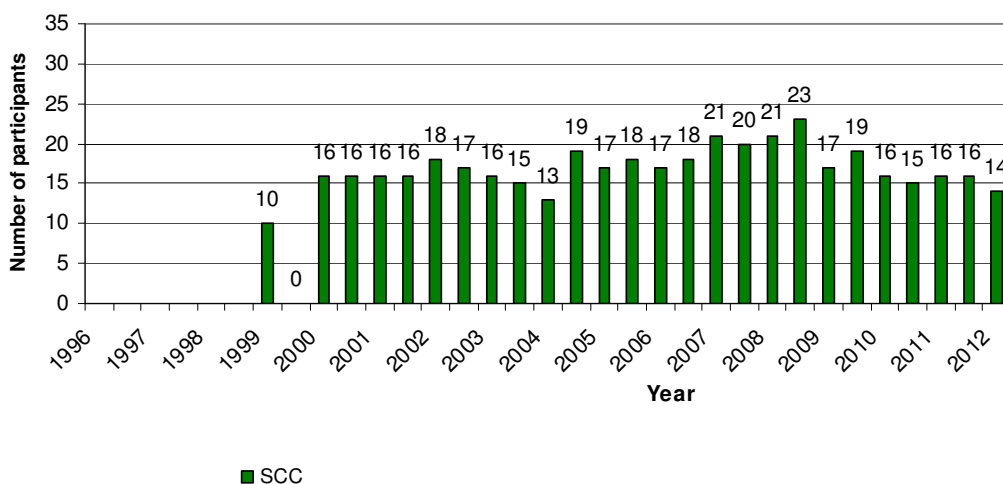Figure 7. Evolution of participation in ICAR PTs for urea.

Figure 8. Evolution of participation in ICAR PTs for somatic cell counting.

Next evolution in the network activities and conclusion

International anchorage of routine labs through the reference laboratory participation in ICAR trials can result in the calculation of the overall uncertainty of analytical results produced individually by labs. This is one important issue for which MASC can develop guidelines for the sake of ICAR laboratories.

As well, beside the evaluation of labs performance in local or national PT schemes the member organisation can evaluate their labs virtually against the international reference produced in ICAR trials through the PT scheme anchorage system develop within MASC. Provided minimum cautions, such a system enables to compare performance with labs of other schemes without any participation in common. This system is explained more in depth in the next presentation and as well guidelines should be developed on that issue.

For the next future prospect is presently made to evaluate the possibility for international PT for routine methods and investigate on the overall accuracy and precision provided by applying centralised calibration using reference materials within ICAR. As well need is for guidelines to define the suitable characteristics of reference material used for calibration and the adequate process to validate and assign

international reference values to RMs and with this respect reference can be made to presentations made in Niagara Falls 2008.

From 2009 ICAR Reference Laboratory Network and surrounding activities run by MASC are elected as the example and the starting base of an innovative IDF-ICAR programme on reference System for somatic cell counting presented in ICAR Session 2010 in Riga.

All these developments foreseen and already partially undertaken will require financial support and time and expertise of key persons. Therefore the organisation and financing of the development and activities must be thought through.

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MA SC

ICAR Sub-Commitee on
Milk Analysis

ICAR Reference Laboratory Network

- Objectives & Stage of Progress in 2012 -

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- INTRODUCTION - GENERAL OBJECTIVES -

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

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

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

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

    > International network of reference laboratories for milk recording
        analytical performances

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ROLES OF THE LABORATORY NETWORK

ICAR Reference Laboratory Network
**an international platform for milk recording**

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

- to provide precision traceability and anchorage to consensual
international "true values" to routine labs via network members

=> analytical data harmonisation   *(PTs, RMs)*

- to develop collaborations / programmes for laboratory purposes
=> Co-operation   *(Education, training)*

Model & explanation provided every year to ICAR member organisations
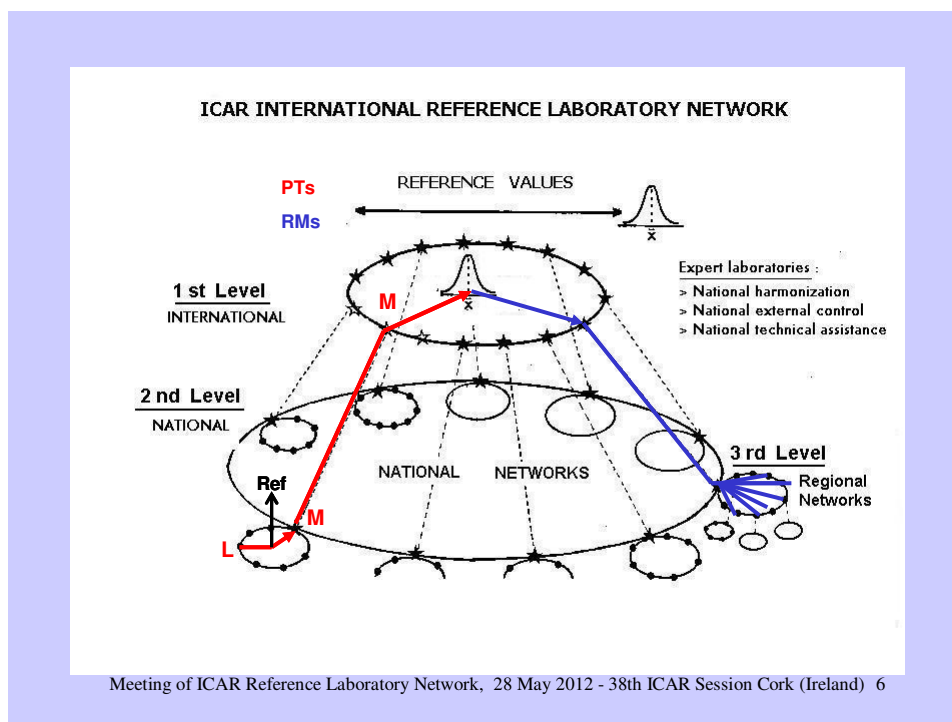
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THEORETICAL STRUCTURE
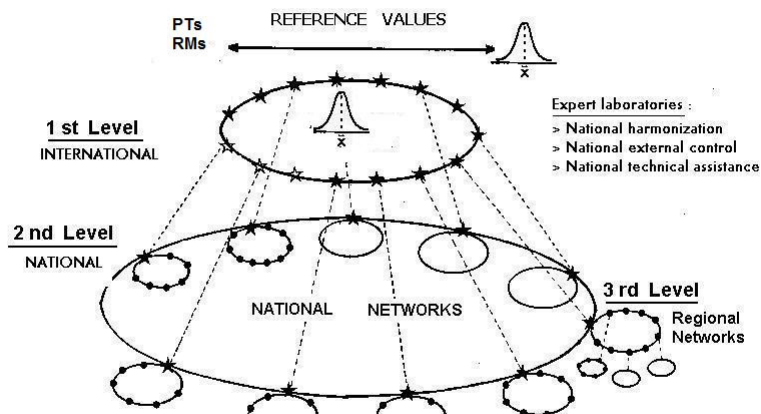
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ICAR INTERNATIONAL REFERENCE LABORATORY NETWORK

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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
Membership in 2011-2012

41 laboratory members from **34** countries as follows:

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

(n) : number of member(s)
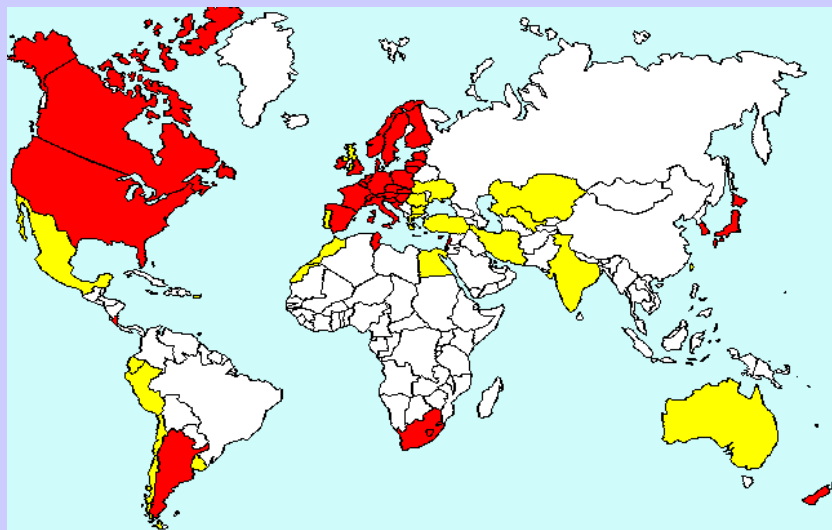
among which :   41 members for cow
                   19 members for goat
                   16 members for sheep

3 new members in 2011 : Croatia, Germany, Japan

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ICAR Reference Laboratory Network
Geographical distribution in 2011-2012

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Evolution of the composition and national roles from 1998 to 2011

YEAR	NRTO	RMS	MLCC	TLT	IAM	EAMI	RAM	NRCA	DHIA	PAYMENT	Other anal.	Members
1998	15	16	13	13	16	1	11	2	2	1	1	23
1999	17	18	17	14	17	1	12	2	3	1	1	28
2000	16	21	19	15	19	1	13	3	5	1	1	33
2001	19	22	19	18	21	3	15	5	6	2	1	35
2002	20	23	19	19	23	8	15	8	11	5	1	37
2003	21	26	19	21	24	12	16	9	14	7	3	38
2003	21	26	19	21	24	12	16	9	14	7	3	38
2004	25	26	18	20	24	14	16	9	16	9	3	38
2005	24	24	17	19	22	13	15	10	15	8	3	37
2006	24	24	17	20	22	14	15	10	15	10	3	36
2007	22	24	18	22	24	17	17	13	17	13	3	38
2008	23	24	18	22	24	17	17	13	17	13	3	38
2009	23	24	18	22	24	17	17	13	17	13	3	38
2010	23	25	19	21	23	17	17	12	18	14	3	38
2011	25	27	21	24	26	19	19	14	19	14	4	41

NRTO = National Ring Test Organiser   RMS = Reference Material Supplier   MLCC = Master Laboratory for Centralised Calibration
TLT = Training in Laboratory Techniques   IAM = Information on Analytical Methods   EAMI = Evaluation of Analytical Methods/Instruments
RAM = Research on Analytical Methods   NRCA = National Regulatory Control of Analyses   DHIA = Dairy Herd Improvement Analyses
Membership = Officially nominated by ICAR National Committees   Payment = Analyses for milk payment

Evolution of the proportions of national roles from 1998 to 2011

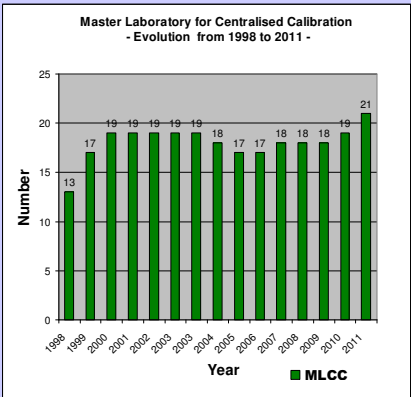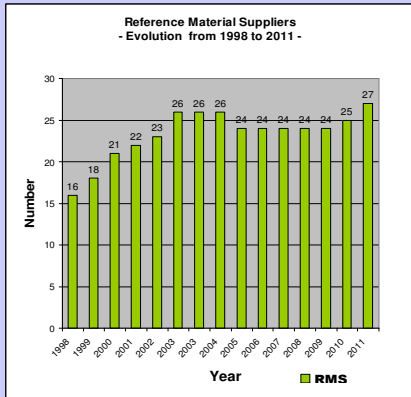| YEAR	NRTO	RMS	MLCC	TLT	IAM	EAMI	RAM	NRCA	DHIA	PAYMENT	Other anal.	Members
1998	68	73	59	59	73	5	50	9	9	5	5	100
1999	63	67	63	52	63	4	44	7	11	4	4	100
2000	48	64	58	45	58	3	39	9	15	3	3	100
2001	54	63	54	51	60	9	43	14	17	6	3	100
2002	54	62	51	51	62	22	41	22	30	14	3	100
2003	55	68	50	55	63	32	42	24	37	18	8	100
2004	66	68	47	53	63	37	42	24	42	24	8	100
2005	65	65	46	51	59	35	41	27	41	22	8	100
2006	67	67	47	56	61	39	42	28	42	28	8	100
2007	58	63	47	58	63	45	45	34	45	34	8	100
2008	61	63	47	58	63	45	45	34	45	34	8	100
2009	61	63	47	58	63	45	45	34	45	34	8	100
2010	61	66	50	55	63	45	45	32	47	37	8	100
2011	61	66	51	59	63	46	46	34	46	34	10	100

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Evolution of membership and missions/activities from 1998 to 2011

ICAR network members - Evolution from 1998 to 2011 -

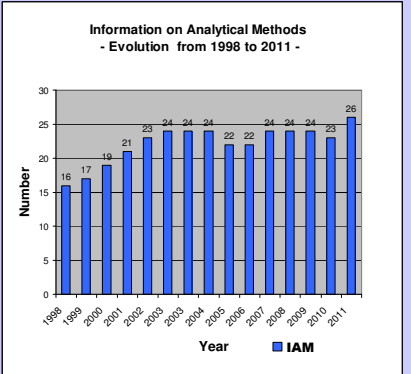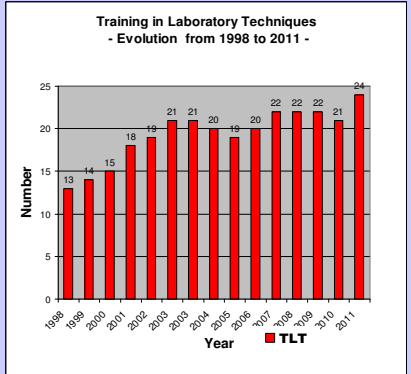National Ring Test Organisers - Evolution from 1998 to 2011 -
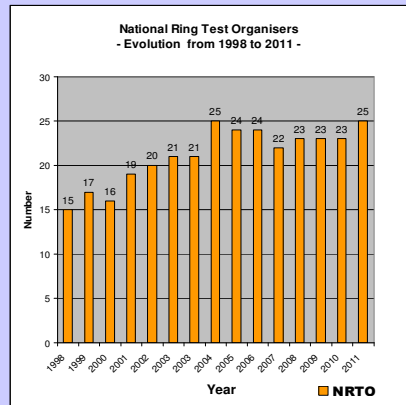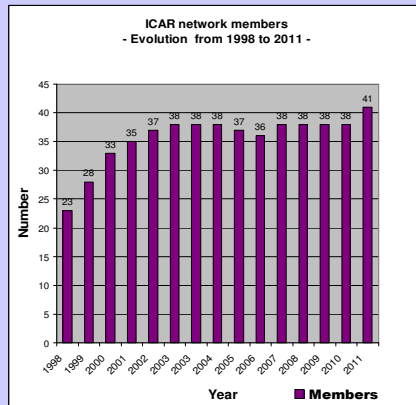
Meeting of ICAR Reference Laboratory Network, 28 May 2012 - 38th ICAR Session Cork (Ireland)11

Evolution of membership and missions/activities from 1998 to 2011

Reference Material Suppliers
- Evolution from 1998 to 2011 -

Master Laboratory for Centralised Calibration
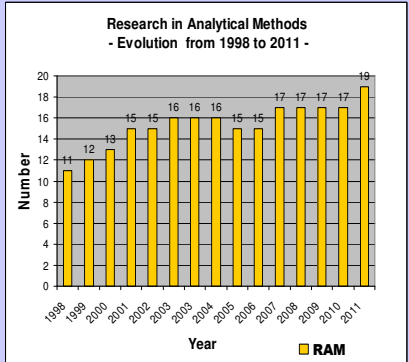**- Evolution from 1998 to 2011 -**

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Evolution of membership and missions/activities from 1998 to 2011

Training in Laboratory Techniques
- Evolution from 1998 to 2011 -

Information on Analytical Methods
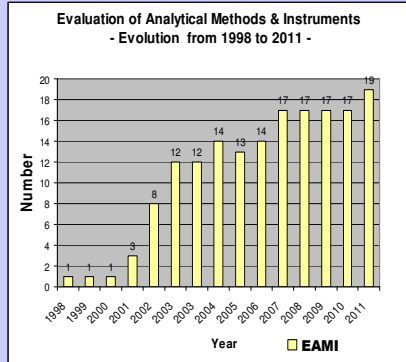**- Evolution from 1998 to 2011 -**

Meeting of ICAR Reference Laboratory Network, 28 May 2012 - 38th ICAR Session Cork (Ireland) 13

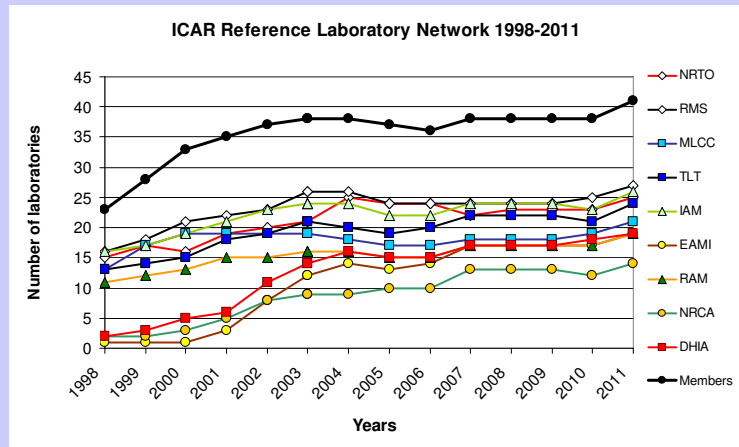## Evolution of membership and missions/activities from 1998 to 2011

Evaluation of Analytical Methods & Instruments
- Evolution from 1998 to 2011 -

Research in Analytical Methods
- Evolution from 1998 to 2011 -

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Evolution of membership and missions/activities - Number -

ICAR Reference Laboratory Network 1998-2011

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Evolution of membership and missions/activities - Percentage -

ICAR Reference Laboratory Network 1998-2011
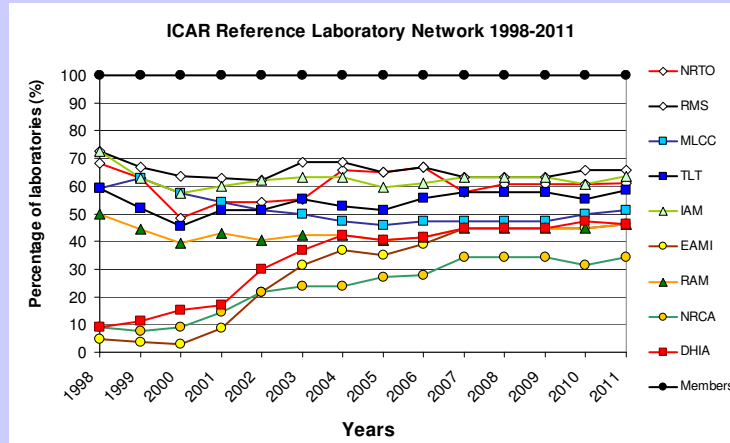
Meeting of ICAR Reference Laboratory Network, 28 May 2012 - 38th ICAR Session Cork (Ireland)16

Coverage of eligibility criteria in 2011

Criteria N	Proportion	nr lab with nr	% lab with N	nr lab with at least N	% lab with at least N
8	100%	7	17%	7	17%
7	88%	5	12%	12	29%
6	75%	4	10%	16	39%
5	63%	3	7%	19	46%
4	50%	7	17%	26	63%
3	38%	3	7%	29	71%
2	25%	2	5%	31	76%
1	13%	4	10%	35	85%
0	0%	6	15%	41	100%

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Coverage of eligibility criteria in 2011

Criteria N	Criteria %	2008	2009	2010	2011
8	100%	13%	13%	13%	17%
7	88%	24%	24%	26%	29%
6	75%	37%	37%	37%	39%
5	63%	45%	45%	45%	46%
4	50%	63%	63%	61%	63%
3	38%	71%	71%	68%	71%
2	25%	76%	76%	76%	76%
1	13%	87%	87%	87%	85%
0	0%	100%	100%	100%	100%

| No criterion		13%	13%	13%	15%

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International interlaboratory proficiency studies

From 1996 :   International proficiency scheme organised by ICAR

Frequency :   twice a year (4 new PT rounds since Riga 2010)

Participants :   13 to 19 members of ICAR Ref Lab Network

Analytical methods :   - reference methods to calibrate routine methods
for fat and protein

- methods for lactose, urea somatic cell counting

Type of milk :   cow milk
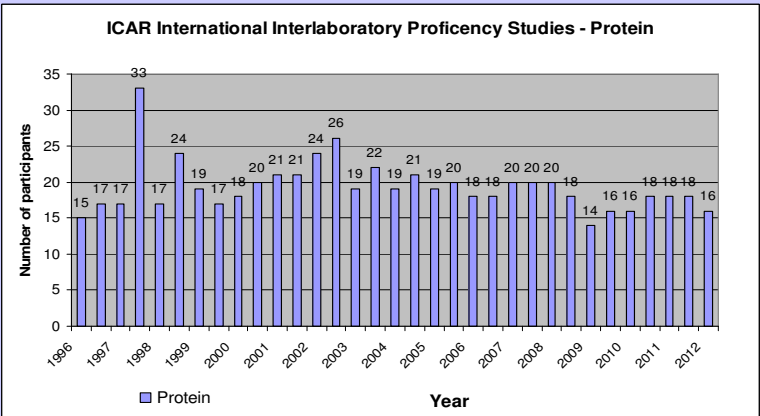
Meeting of ICAR Reference Laboratory Network, 28 May 2012 - 38th ICAR Session Cork (Ireland) 19
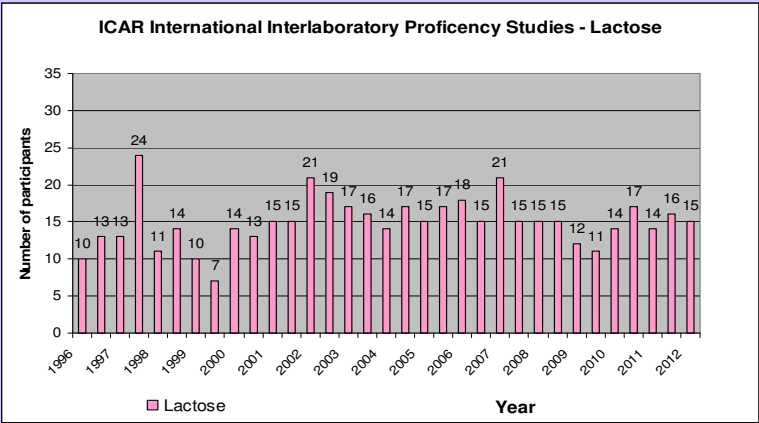
Participation in ICAR PTs - FAT

ICAR International Interlaboratory Proficency Studies - Fat
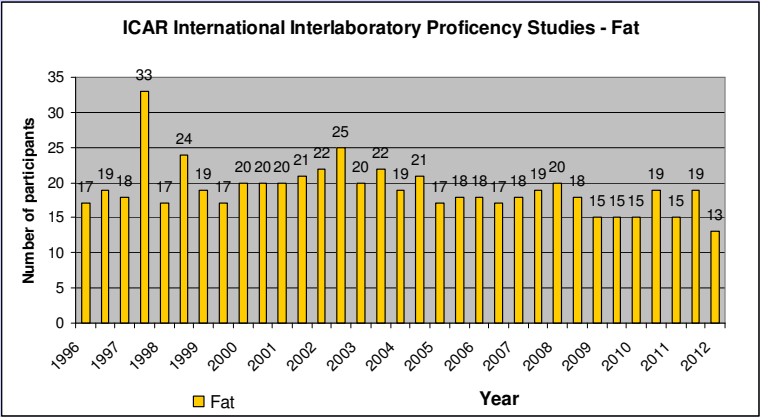
Meeting of ICAR Reference Laboratory Network, 28 May 2012 - 38th ICAR Session Cork (Ireland) 20
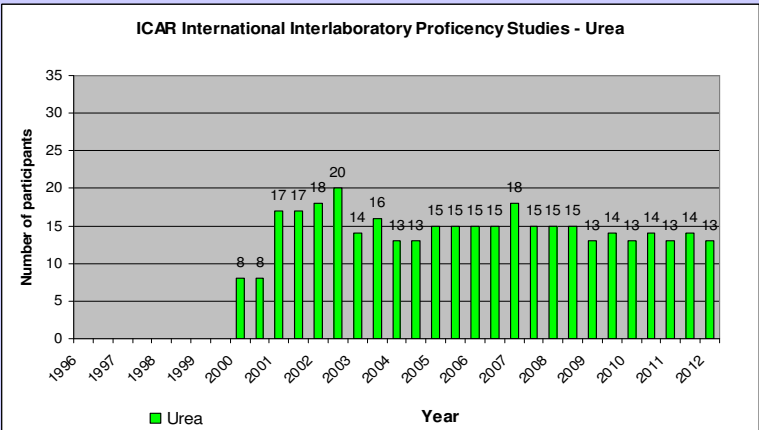
Participation in ICAR PTs - PROTEIN

ICAR International Interlaboratory Proficency Studies - Protein

Meeting of ICAR Reference Laboratory Network, 28 May 2012 - 38th ICAR Session Cork (Ireland) 21
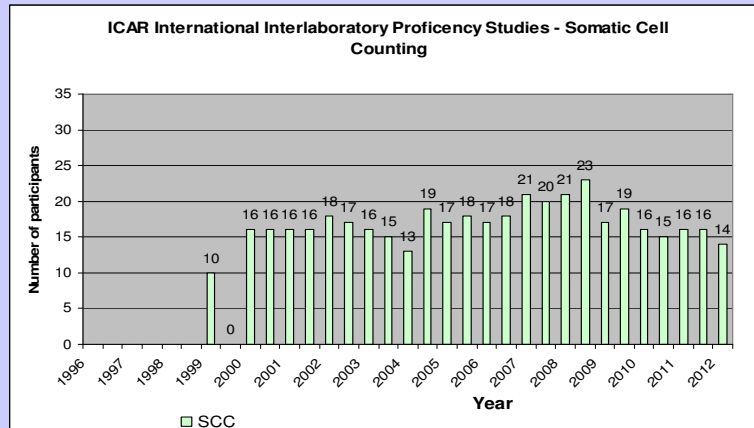
Participation in ICAR PTs - LACTOSE

ICAR International Interlaboratory Proficency Studies - Lactose

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Participation in ICAR PTs - UREA

ICAR International Interlaboratory Proficency Studies - Urea

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Participation in ICAR PTs – Somatic Cell Counting

ICAR International Interlaboratory Proficency Studies - Somatic Cell Counting

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From Sousse 2004, Niagara Falls 2008, Riga 2010
Interlaboratory proficiency studies are promoted for

1- Measuring laboratory performance

2- Measuring result uncertainty

3- Comparing laboratories (assess equivalence)

4- Providing traceability to international reference

5- Qualifying/selecting reference/expert laboratories

6- Assessing/certifying reference materials

In order to built-up consistent reference systems
nationally and internationally

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CONCLUSION ON THE NETWORK IMPLEMENTATION

Nominations by national organisations :

- **Number :** 41 members
3 new members in 2011 : Croatia, Germany, Japan

Need to expand more widely for a worldwide recognition
purpose with new ICAR members
e.g. Latin America, Asia, Russia & CIS

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

International Proficiency Testing schemes :

- Regular participation of about 40-45 % of laboratory network members

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Thank You for your attention !

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International survey on somatic cell counting – Situation of lab network organisation and practices

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Abstract

The parameter of somatic cell count in milk (SCC) is one of the most important indicators for udder health of cows and therefore for milk quality. More than 500 million analyses are estimated to be performed each year worldwide. Due to the great importance of this parameter the ICAR/IDF project on "Reference System for Somatic Cell Counting" ("RefSysSCC") has the aim to enhance and secure analytical equivalence on a global scale.

The project group "RefSysSCC" agreed from the beginning to develop the project in a way as to start from the present analytical situation and to consider all the various analytical solutions adopted for anchoring somatic cell counting in the different countries. To gather that practice information two international surveys were dispatched:

- a first questionnaire was outlined for reference material producers,
- while the second was addressed to the routine laboratories.

14 different reference material providers sent information to the project group on the nature, range and shelf life of their products and 215 routine laboratories from 36 countries of all five continents described the use of reference materials and calibration procedures used to check the automated analytical systems.

After analyzing the large number of answers received, interesting interlinks between different systems and countries could be shown. ICAR Reference Laboratory Network appears to have a strategic role there. Based on this information the project group would like to propose an international analytical architecture, where these connections will be harmonized and used to build up a system for securing traceability in the measurements and to obtain better analytical equivalence worldwide together with all participating stakeholders.

Introduction

The parameter of somatic cell count in milk (SCC) is one of the most important indicators for udder health of cows and therefore for milk quality. More than 500 million analyses are estimated to be performed each year worldwide. This parameter is used in farm management. Breeding programs and it is a criterion for hygiene regulation and milk payment. Due to the great importance of this parameter the ICAR/IDF project on "Reference System for Somatic Cell Counting" ("RefSysSCC") has the aim to enhance and secure analytical equivalence on a global scale. A fair international trade in the dairy sector requires that the results are comparable between laboratories, between countries and between methods used.

The analyses for somatic cell counting are performed worldwide using automated instruments. These instruments must be checked using appropriate quality assurance procedures and reference materials. The international procedures for reference and routine method were recently revised but reference method has a poor performance while certified reference materials are not available and the characterization processes of the secondary reference materials are not standardized. Due to the weakness of the quality assurance tools to achieve a better equivalence in somatic cell counting it is necessary to provide an international structure to anchor the analytical results: it will facilitate to built the analytical traceability. This is the most important goal of the project on Reference System for Somatic Cell. This project group is composed by 28 very active members from 19 countries and 4 continents! They agreed from the beginning to develop the project as to start from the present analytical situation and to consider the different analytical solutions adopted in the different countries

by the laboratories. Each laboratory usually is a part of a group of laboratories that use the same quality assurance tools, e.g. the same secondary reference material or they participate in the same proficiency testing scheme or they use the same guidelines. For the project is extremely important to know "if and how" the different groups are connected.

To gather these practice information two international surveys were dispatched: a first questionnaire was outlined for reference material producers, while the second was addressed to the routine laboratories.

Information gathered on reference material producers

From the two questionnaires, we have received information on 21 different reference material producers for somatic cell 14 from Europe, 4 from United States, 1 from South Africa 2 from ASIA. From the answers received we have listed the characteristics of the different reference materials produced, they consist either in raw milk or heat-treated milk with natural somatic cells but in some cases also with cells from other matrices. They are different in range, number of samples, process of characterization and shelf life.

To establish the assigned value different schemes and techniques are used. Some producers rely on the reference method performed in one laboratory, others combine data from reference and routine method testing, either collected on a small scale or through extensive proficiency testing (see table 1).

Table 1. Replies on characteristics of reference materials for somatic cell counting

CHARACTERISTICS OF REFERENCE MATERIALS FOR SOMATIC CELLL COUNTING	
NATURE	Raw Milk
	UHT Milk
	Natural Cell
	External Cell from other matrices
RANGE	80.000-1.000.000 cells/ml
	200.000-500.000 cells/ml
	150.000-1.300.000 cells/ml
NUMBER OF SAMPLES /LEVELS	2
	4
	5
	10
CHARACTERIZATION PROCESSES	Proficiency Test with Ref.& Automated method
	Reference Method
	Automated and Reference Method
SHELF LIFE	From 5 days to 180 days

An interesting aspect was to observe that some reference material producers compare different type of reference materials and participate in different proficiency testing schemes. It means to create interlinkages and comparison between the different providers. To manage these connections with a scientific statistic scheme is the beginning of an interlinked networked reference system that is the target of this joint ICAR/IDF project. Considering the answers received the experiences of different reference material providers and the last scientific tests, the project group has elaborated two documents:

- o   To describe the requirements of RMs for SCC according the ISO 30-31-34-35
- o   Instruction to prepare RMs for somatic cell

The use of these documents in the analytical dairy sector will facilitate to align and to set the automatic instruments in the same way. This is an other important building block to support the analytical equivalence !

Information gathered from routine laboratories

This second questionnaire was launched in November 2010 to the routine laboratories that analyze somatic cell in milk. The questionnaire accompanied by poster and the introduction letter of the project were translated in 6 languages (EN-ES-FR-DE-IT-RU).

To encourage a positive feed back in number of answers some key person/distributors were charged to hand out the questionnaire. International organizations as ICAR and its Sub Committee Milk Analyses, EU-Reference Laboratories- Milk (EU RL) with their reference laboratories and IDF Head Office facilitated the distribution and the gathering of the answers.

After two distribution sessions final outcome is that we have received answers from the 5 continents, 36 countries that sent 210 laboratories' answers describing 225 instruments functions and operating systems. These results are summarized the table 2 and figure 1.

Table 2. Numbers of laboratories and countries participating in the survey.

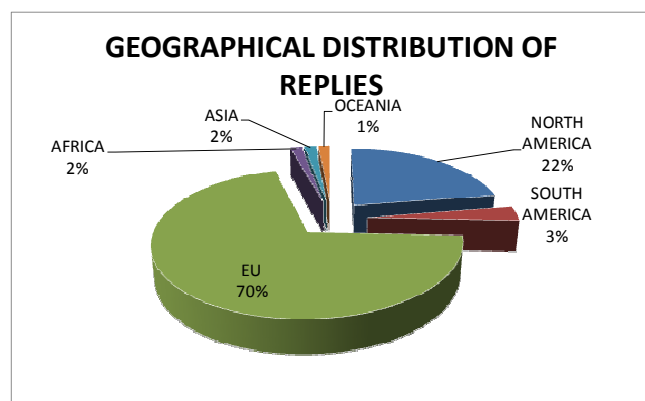|	N of countries	% labs distributions	N of Labs
Total	**36**	**100**	**210**
Europe	26	70	147
North America	2	22,4	47
South America	3	3,3	7
Asia	2	1,4	3
Africa	2	1,4	3
Oceania	1	1,4	3

Figure 1. Geographical distribution of replies received.

From the first part of the questionnaire we have obtained information on the technologies used, the types of instruments, the tools used for control and calibration procedures.

We have registered 5 manufactures that produce instruments to determine somatic cell counting in milk and the technique used are:

Flow cell (FC) 90%, displaying on disc (DD) 7% and charged coupled device (CCD) 3%.

Regarding calibration procedures appeared that the concept of calibration and adjustment is not clear to all the responders. 90 % use reference materials to do the calibration, it means to compare the reference materials with the instrument's answer, and in this group only few laboratories do an "adjustment" that it means to change the slope and bias. 7 % of laboratories do not use any type of reference materials and check own analytical performance with the participation in national and international proficiency tests. 3% did not answered.

Regarding the preservative the most used is bronopol (95% of laboratories) in different final concentration from 0.02% to 0.1%.
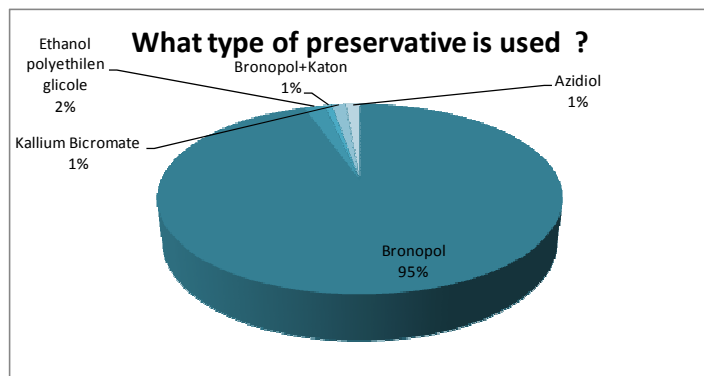
Figure 2. Type of preservative used.

The three main categories of reference material used are natural raw milk (64.25 %) heat treated milk (21.72) and skimmed/half skimmed (3.16%). 1 laboratory uses lyophilized milk, 1 frozen and 10 % don't use reference materials.
The synthetic particles 3% were not considered reference materials.
An other interesting information is that 9% of laboratories use either reference material with natural cells or synthetic particles. 12% of labs use reference material from different providers. This information is confirmed from the answer received for question 5.

Figure 3.	Distribution of type of Reference Materials used.

To the question number 5 "Is the reference material prepared in your laboratory or is it provided by a RM producers ?" 78% of laboratories use a RM provided by a producer 9% didn't answer and 3 % don't use RMs. 10% of the laboratories use more than one reference material.
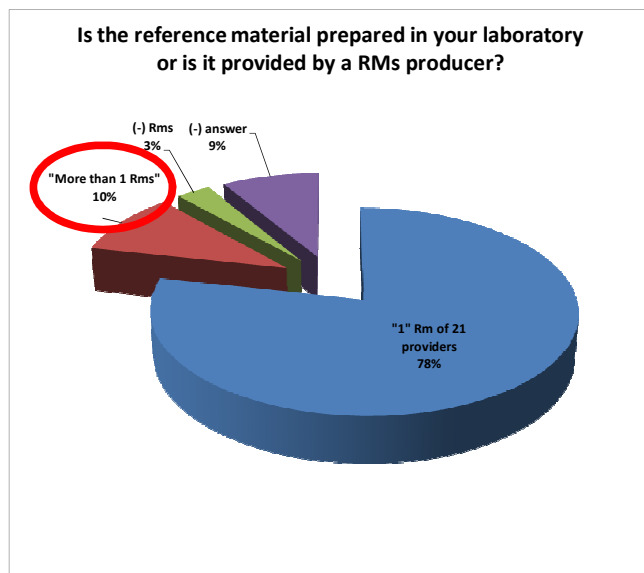

Figure 4. Distribution of the reference materials provided or internal production.

Some of the laboratories that don't use reference materials, check the instruments performing reference methods each month on one sample of about 400 000 cells/ml and compare with the national laboratory, other use only synthetic particles, 2 labs don't use any type of control. The labs that didn't answer or don't use RMs participate in an international or national proficiency test.

The laboratories that calibrate the instrument specified the frequency too. To the question 6 "Calibration set and frequency of calibration". 73% of laboratories conform to the standard ISO 13366-2 because they have indicated to do the calibration once per month. 20% of laboratories check the intruments from 3 to 12 months and 7% didn't answered.
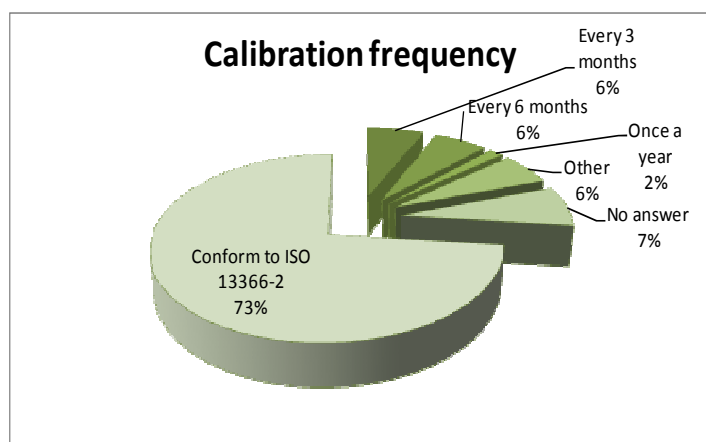

Figure 5. Calibration Frequency.

The range of the calibration sets provided by the different reference material providers were divided in 7 categories and 44% of laboratories check the range between 100.000-1.000.000 cells/ml and 44% between 100.000-1.600.000 cells/ml. Some laboratories declared that even if they use a large calibration set (e.g. 100.000-1.800.000 cells/ml) they optimize the accuracy around 400.000 cells/ml The question 7 "What are the tolerances for eventual adjustment?". Only 30 % of laboratories answer to this question (64 laboratories). Of these 64 laboratories 86 % of laboratories adjust the instrument or call the technical assistence if they are not in the range for slope between 0.95-1.05.
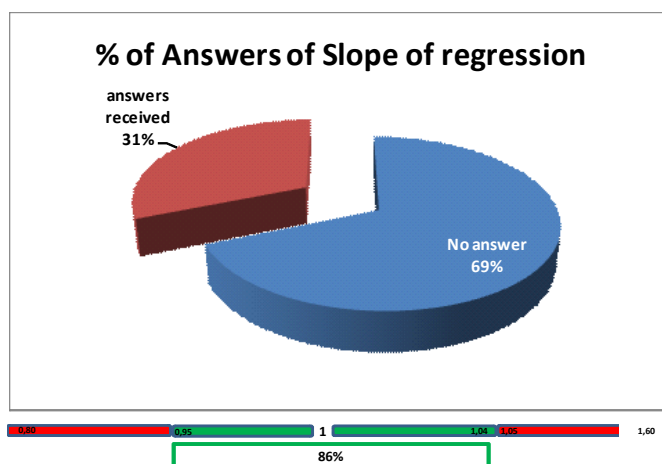

Figure 6. Percentage of laboratories answering on slope adjustment.

Regarding the mean relative bias only 27% of labs answered. 50 % of them (28 laboratories) declared to accept a maximum value of 5 %.

Interlaboratory comparison and interlinkages

Question 8 was on "Interlaboratory comparison" on the National or International level. This question was very important to mapping out the distribution and interconnections between the different groups of laboratories. From the previous questions we have seen that some laboratories don't use reference material but prefer to participate in proficiency testing schemes to verify the performance of own instrument/s. 206 laboratories answered according Figure 7 where we can see 104 laboratories

participate in national interlaboratory comparison, 19 in international and 72 in both schemes. 11 laboratories don't participate to any comparison.

Figure 7. Number of laboratories participating in PT schemes.

The fact a laboratory participates in different proficiency testing schemes means that it is a part of two different populations of instruments. As an example in the figure 8 are reported the positions of the same laboratory in different proficiency testing schemes. This parallel participation highlight different bias of own laboratory against the assigned value, and sometimes it is not easy to explain and to understand the reason of it. Some explanations of this picture can be:

- the three different groups of laboratories use different reference materials to calibrate the instrument. The consequence of it could be that the instrument is more accurate in a particular range e.g. around 400.000 cells/ml instead of 1.000.000 cells/ml
- the range and number of samples offered by the provider of proficiency test are different and the lab performance and the overall statistical output and conclusion can be different
- the matrices and cell of the samples in the three groups are different (raw milk, UHT, natural cell, external cell) the instrument's answer could be different
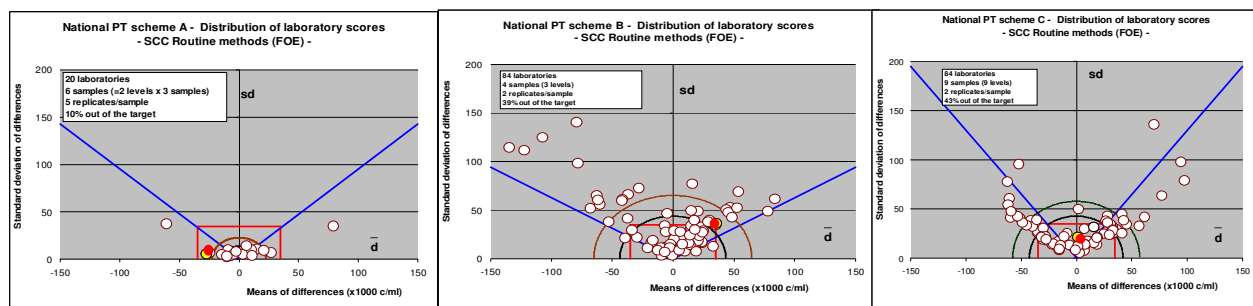

Figure 8. Position of a laboratory that participates simultaneously in different PT schemes.

All these situations need to be harmonized and this is in the scope of the project of Reference System for Somatic Cell.

From the answer received is clear that in the real analytical situation the laboratories participate in national and international proficiency tests with different ranges of concentration Figure 9 and Figure 10.
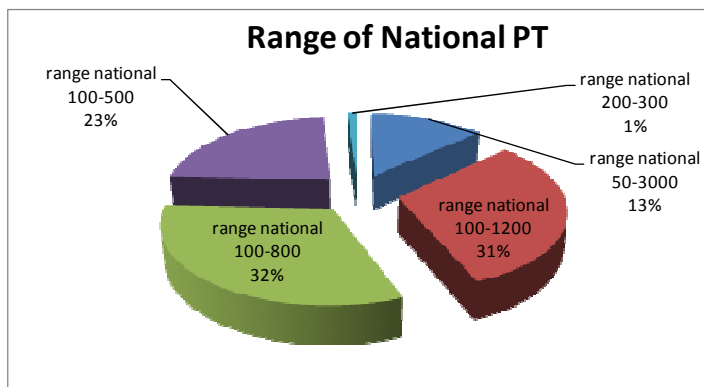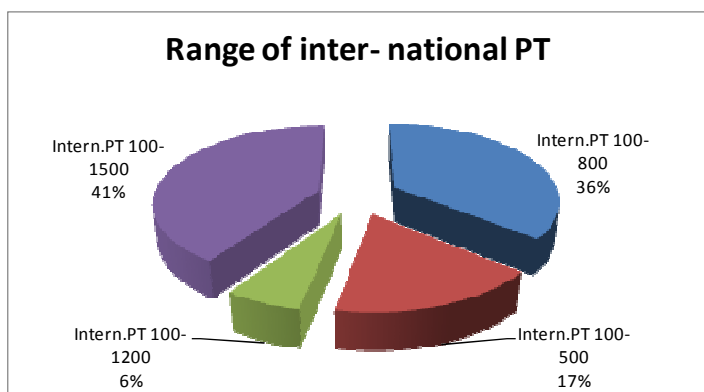
Figure 9. Range of the national PT schemes.

Figure 10. Range of the international PT schemes.

83 % of laboratories participate in scheme with a range from 100.000 to 800.000 cells/ml.
It was very much interesting to draw the map of the different national or international connections of the different countries. Each country has one or more PT schemes and we have focused the attention on the main connections of each continent with the other:

- America: There are interconnections between of Argentina with Bolivia and Brazil, a connection between Canada and USA (Figure 11).

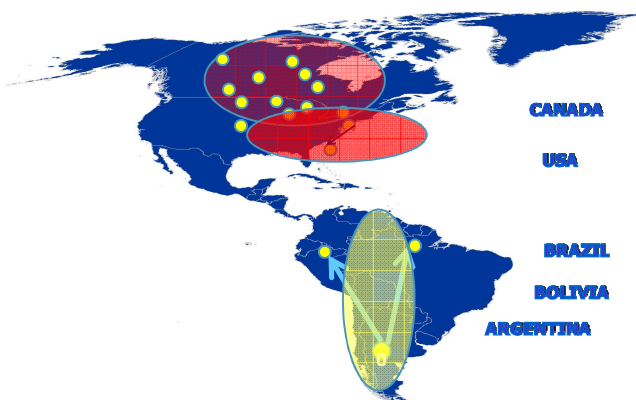Figure 11. Connections between the countries in America.

America is connected with Europe through Argentina and to Oceania through USA.
The project could provide the possibility to study the data of Argentina, USA and New Zealand and to create a linkage between the three continents (Figure 12).

International survey on somatic cell counting – Situation of lab network organisation and practices

Figure 12. Connections between America and other continents.

- Asia: We have received the answer from Israel and Japan and both of them are interconnected with two different countries in Europe. They participate in two different European PT schemes. Israel takes part also in the ICAR proficiency test. The potentiality to compare these two sets of results could give the possibility to interlink Japan and Israel (Figure 13).

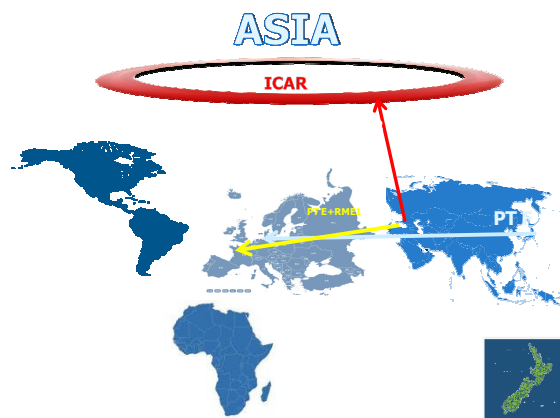*Figure 13. Connections between Asia and other continents.*

- Oceania: New Zealand was connected with Europe and recently (communicated during the ICAR session in Cork) is connected with USA. The comparison of these two connections initially with Europe and now with USA could be an important information for the project group (Figure 14).
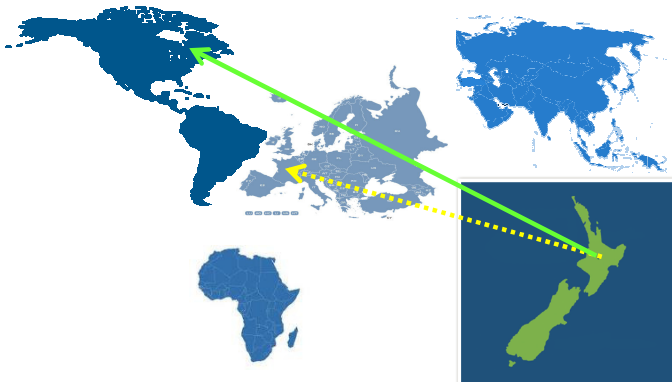

Figure 14. Connections between Oceania and other continents.

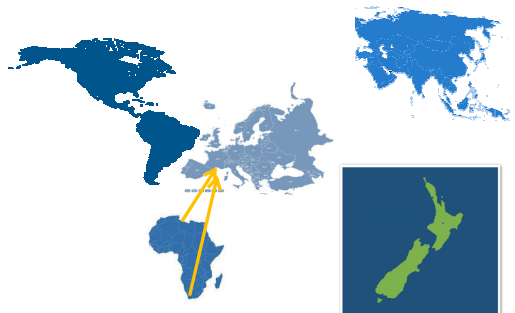- Africa: Two laboratories are connected with Europe:

Figure 15. Connections between Africa and other continents.

- Europe: From Europe we have obtained a very complete picture thanks the 153 received answers from 26 countries. Apparently the map appears complicated because many countries are connected each other trough national and international proficiency tests and several reference materials. We have counted 8 international proficiency schemes and 11 countries are connected with ICAR PT. The further studies of these connections and on the data obtained in the different schemes will be the great adding value of this project.
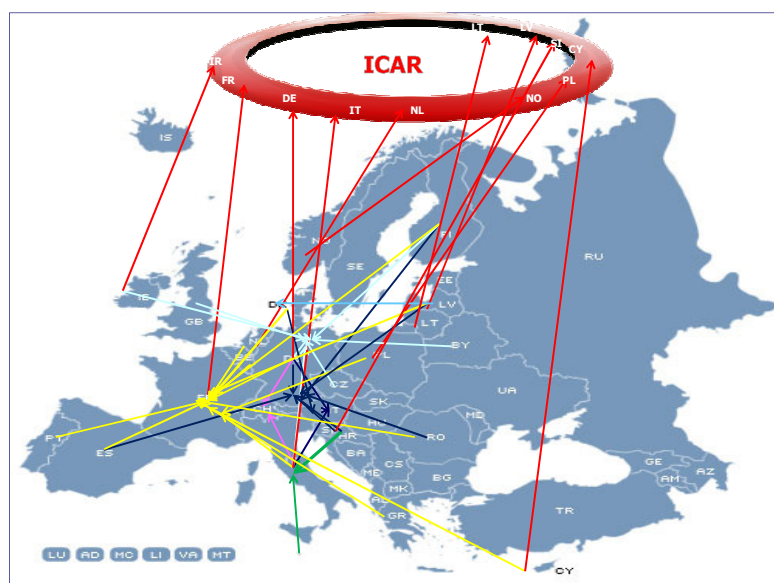
Figure 16. Connections between Europe and other continents.

Now that we have the picture the project group can ask the data to the laboratories that want to share anonymously to test the probabilistic approach to assign a quality index to the different proficiency testing schemes.

Thanks to these surveys now we have the basis to try to increase and improve the interconnections to built up a robust global Reference System for SCC. ICAR as international organization appears to have a central role in Europe with all the potentiality to expand in the other continents. All the contacts received from this survey will be very useful to disseminate the progress on this project and now there is the possibility to focalize the attention on the laboratories that use to interlink more than one system, to analyze the data and to plan a pilot study for testing the reference system for somatic cell.

Conclusion

The map obtained needs to be completed but all these pieces of information describe clearly that the present analytical picture requires to be harmonized and to be anchored to an international structure to built up analytical equivalence in the determination of somatic cells worldwide.

The possibility to be a part of a harmonized international system will give the possibility to be traceable and to demonstrate to the third parts the adoption of a neutral analytical quality assurance tool. This international analytical platform will offer also the possibility to share information and practical analytical solutions to maintain anchored the determination of this parameter.

The interested stakeholders with own participation will link own solutions to the international scenario and their results will be comparable on global scale.

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International Survey on Somatic Cell Counting- Situation of lab network organization and practices

Silvia Orlandini AIA-Laboratorio Standard Latte

on behalf of the

IDF/ICAR Project Group

**ICAR 2012
Cork (IR)**

What's the PG Ref Sys SCC?

It is a PG under the aegis of ICAR and IDF

- **28 Members**
- **19 Countries**
- **4 Continents**

It would like to promote "equilvalence" in SCC counting

Why have we started from SCC ?

SCC
economical aspects

- Widely used test : > 500 000 000 test/year
- Indicator for udder health status (Farm Management)
- Criterion for hygiene regulation (Food Legislation e.g. EU 853/2004) and raw milk payment
- Breeding programs

More reliable SCC building a reference system
⇨ The best way forward !

In the global dairy market, operators need standards for milk quality control

- International trade requires analysis and quality control results
- Results need to be comparable between labs, between countries

Location
Methods
Time

Everywhere ! Every method ! Every time !

How to realize Ref.Sys.SCC ?
Main steps........

- Identify existing « **local analytical systems** »
  - Reference materials' specifications ⇨ RM providers
  - Calibration practises ⇨ RM users (routine labs)

AFTERWARD

- Guarantee quality of reference and routine data (competence of contributing labs)
- Develop a data processing model
- Enhance a pilot study

Why have we to identify different analytical systems ?

BOTTOM UP APPROACH!

System A

Reference System
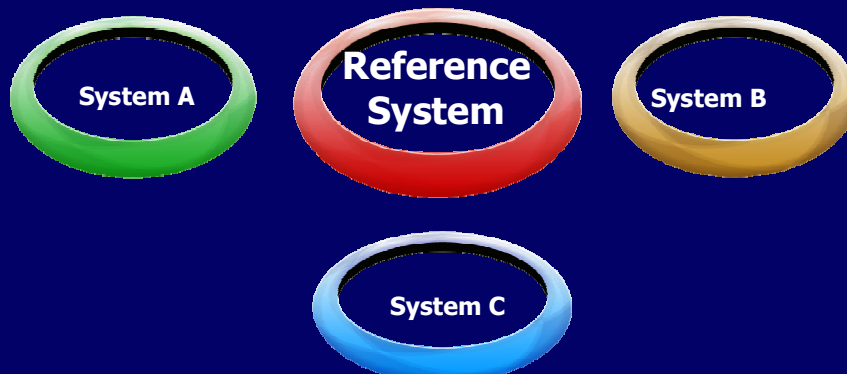
System B

System C

Identified interlinkages between existing local reference systems can form the basis for a 'bottom up" approach

Optimize and interlink the existing systems and evaluate eventual bias

⇨ **To become gradually a global Reference System !**

How to collect data……..

BOTTOM UP APPROACH!

International surveys !!!
Reference material producers

Routine laboratories

In 2010 it was launched and we have received answers from

13 providers
11 from Europe
2 from AMERICA

International surveys !!!
Reference material producers
Routine laboratories

- **Matrix**
  - raw milk
  - heat treated milk
  - natural cell
  - external cell
  - bulk/single cow milk

- **Range**
  - 80.000-1.800.000 cell/ml
  - 150.000-1.300.000 cell/ml
  - 200.000 – 500.000 cell/ml
  - 80.000 - 800.000 cell/ml

80.000   1.800.000

- **Number of levels and samples**

- **Different characterization processes**

Reference method
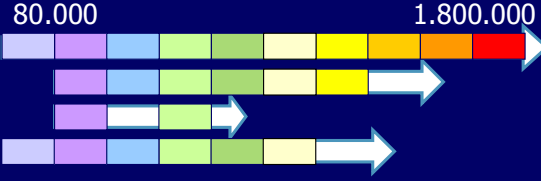
Routine methods

Proficiency Test

Assigned value

International surveys !!!
Reference material producers
Routine laboratories

From these results……

The PG Ref Sys SCC is outlining documents:

- To describe the requirements of a RMs for SCC

- Instruction to prepare the Rms for SCC

International surveys !!!
Reference material producers
Routine laboratories

BOTTOM UP APPROACH!

206 labs

225 Instruments

36 Countries

5 Continents
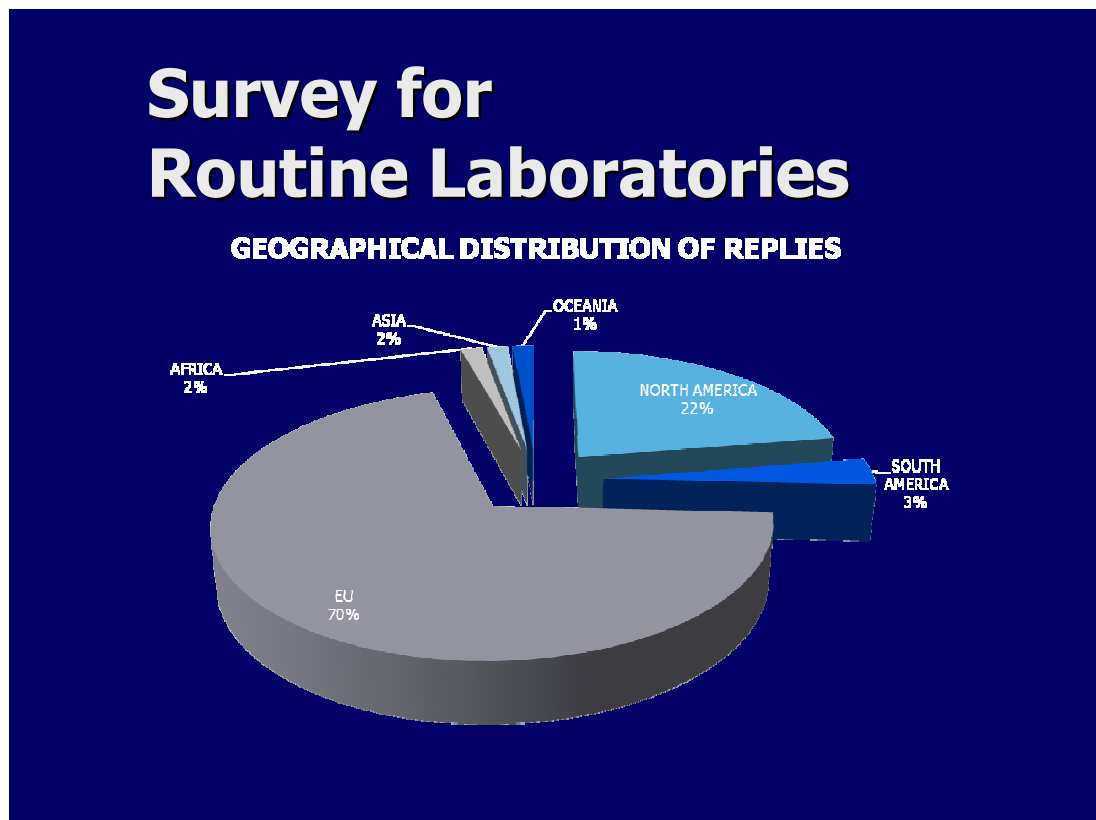
International surveys !!!
Reference material producers
Routine laboratories

1. **Please, list the different types of instruments used for SCC routine testing in your laboratory**
2. **Do you calibrate the routine method?**
3. **What kind of reference material is used for calibration purposes?**
4. **What type of cells are used in the reference material?**
5. **Is the reference material prepared in your laboratory or is it provided by a RMs producer?**
6. **Calibration set and frequency of calibration?**
7. **What are the tolerances used for eventual adjustment?**
8. **Do you participate in interlaboratory proficiency studies ?**

Technology used

Manufacturer 4
6%

Manufacturer 5
1%

Manufacturer 3
1%

Manufacturer 2
6%

Manufacturer 1
86%

Techniques used

DD
7%

CCD
2%

FC
90%

DD= Displaing on Disk
CCD = Charge Couple Device
FC= Flow Cell
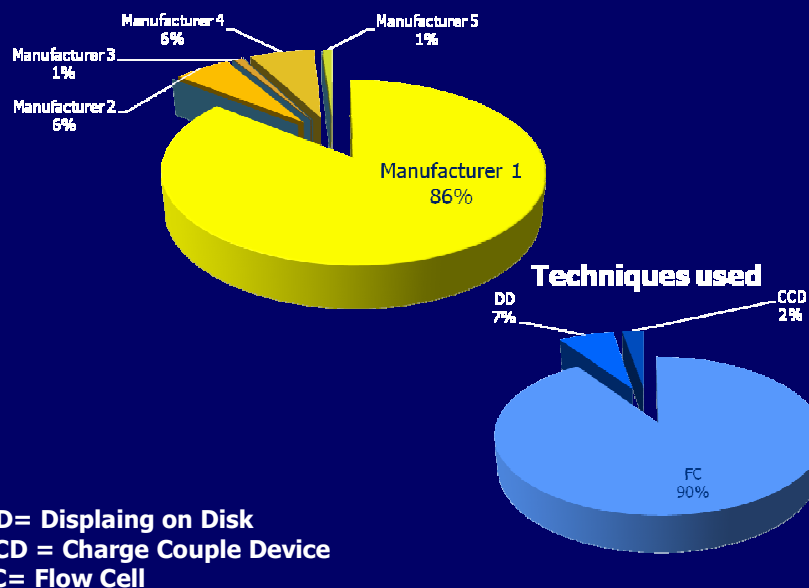
Reference Materials used

Is the reference material prepared in your laboratory or is it provided by a RMs producer

21 Providers ...in the first survey only 13

	CH-CZ-3DE-DK-ES-FR-IR-IT-LV-NL-PL-2SI	**15**	**(11)**
	AR-2CN-USA	**4**	**(2)**
	JAPAN	**1**	**(X)**
	NZ	**1**	**(X)**
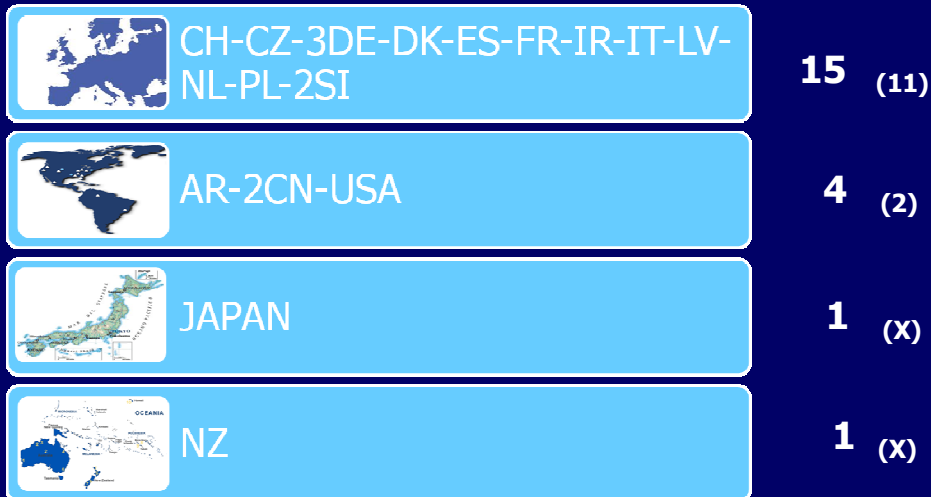
Calibration Procedures

Do you calibrate the routine method ?

No 16%

Yes
84%
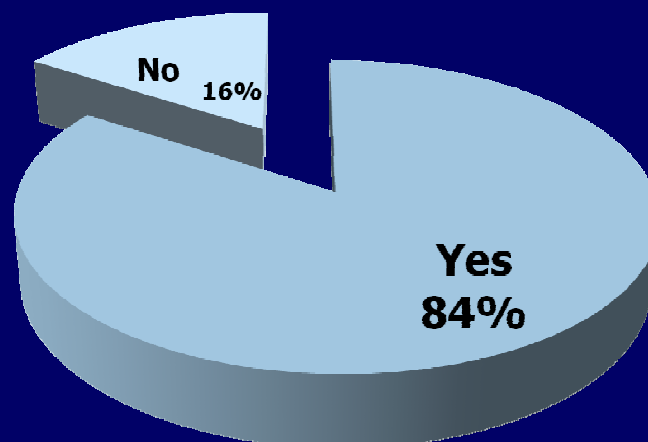
What kind of reference material is used for calibration purposes?

Calibration set and frequency of calibration?

International survey on somatic cell counting – Situation of lab network organisation and practices

International survey on somatic cell counting – Situation of lab network organisation and practices

INTERCONNECTIONS
Interlinked Labs !

INTERCONNECTIONS

> **206 labs 484 connections**

> **21 labs from 14 countries is worthwhile to contact because use more than one RMs or participate in more than one PT scheme !!!**
> **7 are ICAR LABS**

> **4 countries seems to be isolated from the international scenario:**
> **Participate only to one system**

SUM UP

To evaluate the functioning and robustness

Global Reference System
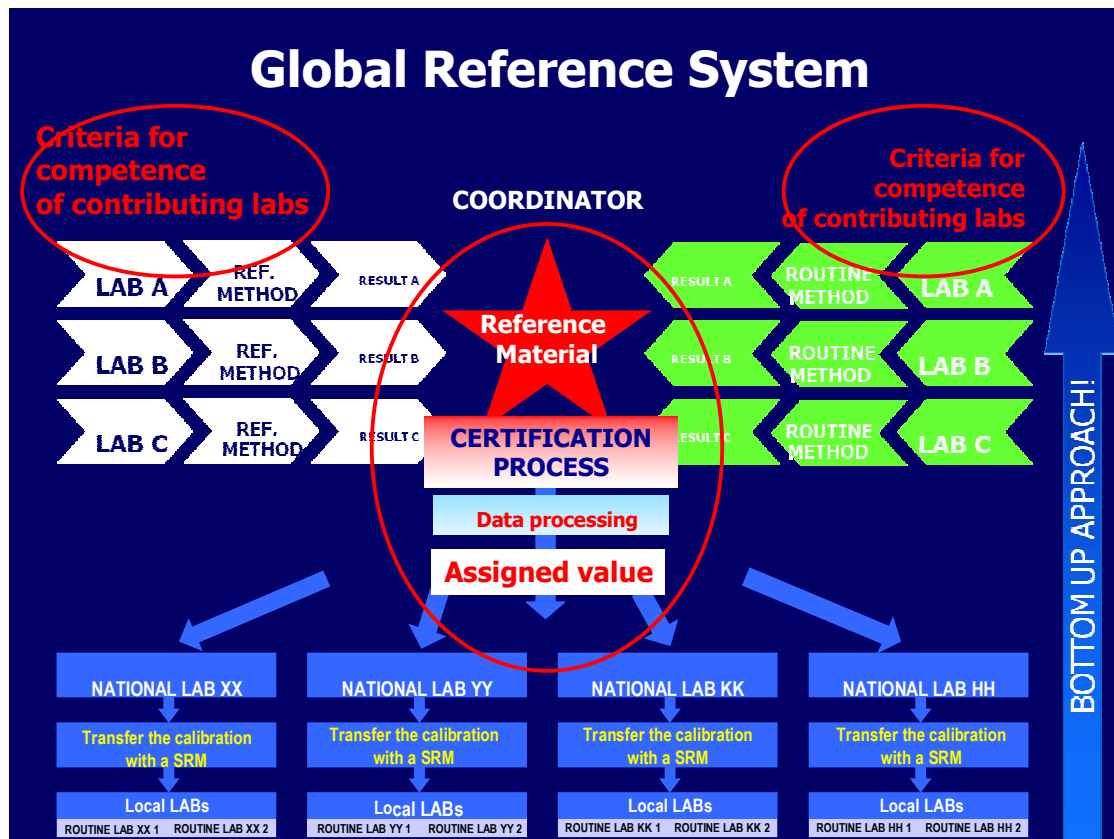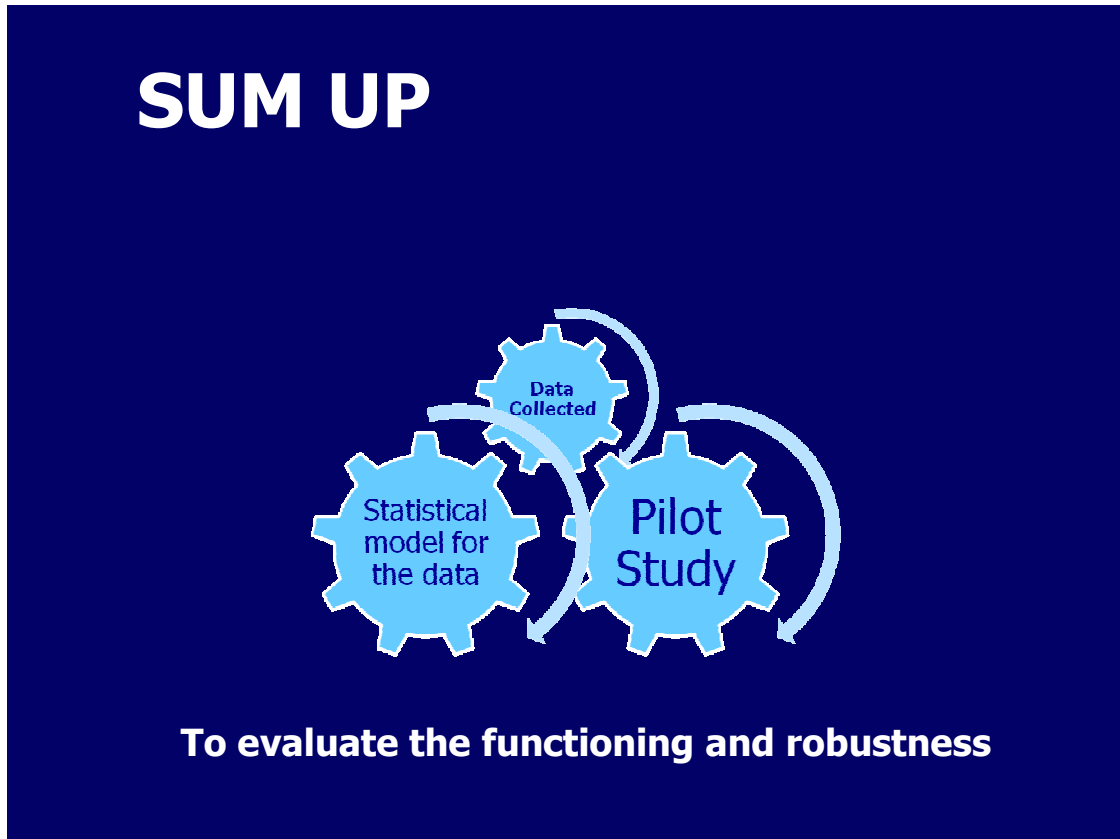
PARTIAL VIEW

To avoid to have a partial view

To avoid to have a partial view

GLOBAL INTERCONNECTIONS PT SCHEMES

Ref.Sys on SCC

For further reading...

- www.fil-idf.org   (working area)
- Newsletters
- Accred Qual Assur (2011) 16:415–420 DOI 10.1007/s00769-011-0797-7
- IDF Bulletin 427/2008

Thank you for your attention !

on behalf of the
IDF/ICAR Project Group

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19 Countries
28 Members**

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PT scheme interlinkage and international laboratory anchorage

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Abstract

The quality of milk recording analytical data is tightly related to the quality of the reference values used by laboratories to calibrate routine testing methods. However the reference methods used by routine laboratories allow differences occur between laboratories within reproducibility ranges. Laboratories's biases as shown in interlaboratory proficiency testing (PT) studies can appears not negligible. Evidence is given that the reference values of a national laboratory network is valid for the group itself but can differ significantly from the reference of other networks depending on analytical methods and PT study conditions. For the sake of the analytical harmonisation within ICAR a general model for PT scheme interlinking is proposed. Laboratory comparison between different independent PT schemes are made possible as well as evaluating lab performance against an international reference as for instance given by ICAR reference laboratory network. Preliminary cautions prior to implementation are given and plea for harmonisation in PT organisation made. Examples from national and international PT trials using somatic cell counting data are presented.

Keywords: milk recording, laboratories, harmonisation, quality assurance, reference, anchorage, network

Introduction

Several hundred millions animal milk samples are analysed every year in milk recording laboratories in the ICAR world. This is rendered possible only by using automated rapid methods mostly based on mid infrared spectroscopy for milk composition and fluoro-opto-electronic methods for somatic cell content. To obtain reliable results these methods so-called routine methods must be regularly calibrated against reference methods and be submitted to regular quality control according to standards and guidelines at the level of the laboratory.

These two last decades have seen a huge internationalization of animal genetic trade (semens, embryos, animals) and genetic evaluation for which the quality of animal performance measurement is of utmost importance so as to allow fair comparison between countries or organisations.

This has justified to ICAR to implement an analytical quality assurance (AQA) system to assure equivalence within and between member organisations thus provide confidence to stakeholders. This system was described in 1994, launched in 1996 then developed progressively till today. It relies on harmonisation of laboratory practices and methods used by laboratories and providing the mean of evaluating laboratory performances within an international reference laboratory network.

The key points of the system are that, through regular international proficiency testing schemes, it allows the reference laboratories

- to evaluate own individual precision and accuracy (bias) for reference methods used against an absolute reference defined by consensus as the average of the results of the group of participating reference laboratories,

- to provide the routine laboratories they monitor with the accuracy traceability to the ultimate truth and the possibility to estimate the real overall uncertainty in the global ICAR system.

Nevertheless if national or regional systems can function separately there is no indication on how effective is the analytical data harmonisation within ICAR. Hence this is the role and the responsibility of ICAR to develop and implement a method that permits to detect and possibly quantify discrepancies between national / regional systems.

Relativeness of reference results and choice of an international reference

A key issue of the ICAR AQA system relates to the so-called reference values used to assess both labs performance and routine methods calibration. Requirement is that they be obtained by internationally standardized reference methods or be standardized methods accurately anchored to the latter.

The exactness of an analytical method is relative since different results lying within standard limits can be obtained for a same milk sample using the same method. Indeed so-called precision figures – repeatability and reproducibility – have been statistically estimated through interlaboratory evaluation method studies according to ISO 5725 and constitute the range of normal observable performances and consequently fix limits for quality control :
- repeatability, r, the largest range not to be exceeded in 95% of cases between duplicates in identical analytical conditions (same laboratory, method, technician, within the closest time),
- reproducibility, R, the largest range not to be exceeded in 95% of cases between duplicates in different conditions (laboratory, device, technician, time) using the same method.

These values enable to establish limits in laboratory performance evaluation by PT and internal laboratory quality control.
From these values can be derived the respective 95% confidence intervals $\pm 2s_L$ and maximum observable ranges L = 2.8 s_L between two laboratory means thanks to the relationship $s_L^2 = s_R^2 - s_r^2$ (Table 1). Those limits indicate possible not negligible difference between laboratories that are then spread over the whole of routine labs through calibration.

Table 1. Range L and limits +/-2 sL for laboratory means derived from standard r and R according to ISO 5725.

Component	ISO	sR	sr	sL	R	r	L	+/-2 sL
Fat	1211	0,02	0,014	0,014	0,056	0,039	0,040	0,029
Protein	8968	0,018	0,014	0,011	0,050	0,039	0,032	0,023
SCC rel (750.10^3 c/ml)	13366-2	6%	3%	5%	17%	8%	15%	10%

Such L or +/-2 sL ranges of possible (accepted) occurrence cannot be found acceptable for every use with regard to the trade value of components which makes critical and questionable the traditional in-house calibration and would plea for more collaboratively obtained reference through centralized calibration (O Leray 2008). Indeed numerous figures observed in interlaboratory proficiency studies illustrate that still larger biases can occur (Figure1).

The same example shows also that in the alternative of centralized calibration the calculated reference (averages) of different groups of laboratories, may differ significantly (e.g. national vs international labs labs larger than 0.01 % protein) whereas in that example ICAR reference provides appropriately the more suitable central position for the reference. Similarly large discrepancies (relative mean biases up to 5-8%) are regularly observed between such groups in somatic cell counting proficiency studies.

Moreover beside reference methods, so-called "secondary reference methods" are permitted by ICAR provided tight anchorage to the international reference methods. For instance case is for fat by the butyrometric method (Gerber) for which national standards exist but no international standards. Ways to relate to ISO 1211 may vary significantly and induce different trueness depending on countries or region (pipette volume, butyrometer calibration, reagents).

66 participants   sd
10 samples in duplicates
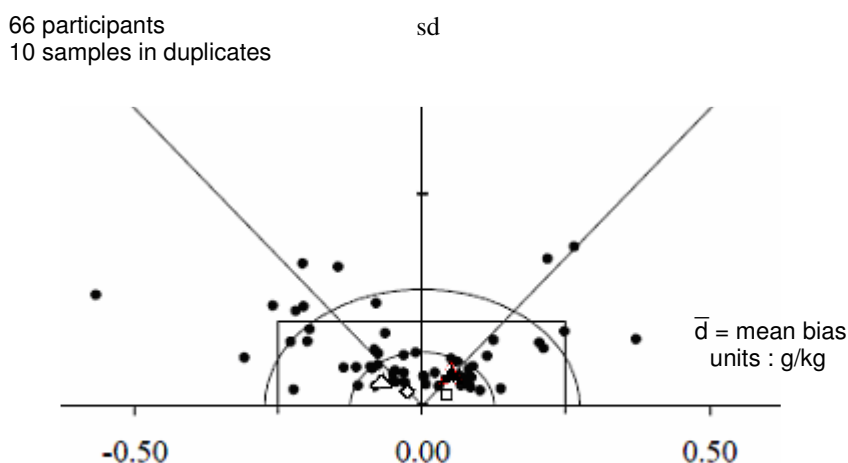
Figure 1. Example of lab score distribution in proficiency study for protein by Kjeldahl method (square ☐ = country labs ; triangle △= foreign labs ; diamond ◇ = ICAR ref labs)

The here above example and many others illustrated by PT studies, supported by the fact that PT scheme conditions vary and may bias the proper estimation of the reference (unequal and irregular participants numbers, different instruments, calibration material, reagent suppliers), plea for the choice of a unique reference defined at the international level by consensus.

Comparing and assessing laboratories on a same unique scale

The ICAR AQA system requires routine laboratories to participate in local proficiency testing (PT) schemes in their countries or regions so as to evaluate and improve performance if needed. In such studies the reference are calculated as the mean of participating laboratories after excluding abnormal (outlier) results. As a result routine laboratories of difference region (different PT scheme) cannot compare to same reference and it happens that two good performing labs of distinct regions do not agree on results when analysing same samples. This can stem from possible differences (see above) in the reference calculated in the respective scheme but it is unknown how similar or close they are if there is no liaison between scheme and no physical link implemented to measure reference similarity.

O Leray (2008) presented a method of PT scheme linkage and anchorage through the common participation of a laboratory in the both trials between which comparisons are wished. The methods addressed single level comparison and applicable by extension to reference methods where there is theoretically no level effect on the bias (lab-reference) which is supposed constant throughout the concentration range (e.g. ISO 1211 for fat). For other methods where a relationship can exist between the bias and the level (e.g. ISO 13366-2 for SCC) need is for another method. Here is described a general method that it is aimed to apply in the on-going joint IDF-ICAR project of Somatic Cell Counting Reference System (Baumgartner & Bijgaart, 2010).

The general principle consists in a way of calibration of the PT scheme(s) to that one chosen as the ultimate reference. To achieve that, a laboratory participates in each of the trials with analysing the samples of each scheme and it is understood that there is no change happened in the method setting it uses in both sample tests performing. Then a relationship F_A allows to predict the reference of scheme A using the results X of the liaison laboratory with the sample set of scheme A and similarly another relationship F_B to predict the reference of scheme B using the results Z of the liaison laboratory with the sample set of scheme B.

Combining the two relationships as $F_B \circ F_A^{-1}(x) = F_B[F_A^{-1}(x)]$ permits to align the reference of scheme A on reference of scheme B then to used the so-calibrated reference as a new reference for a virtual performance assessment. Where the international ICAR scheme is used for scheme B one can speak of a virtual international evaluation.
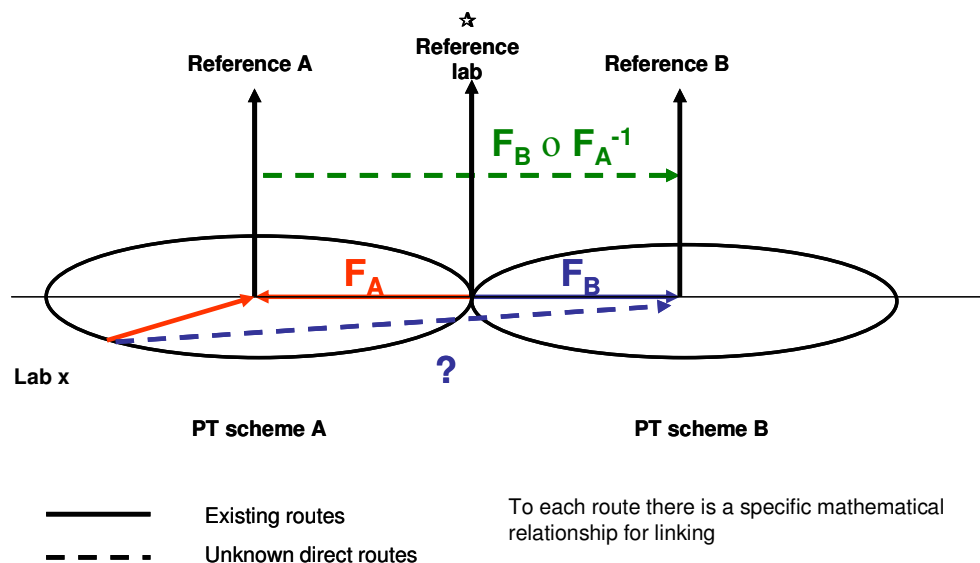
Figure 2. Bridging between two PT schemes by a liaison laboratory (★) and reference alignment of Scheme A to Scheme B.

The nature of the relationships F_A and F_B can be defined by prior polynomial regression optimization through minimizing the regression residual standard deviation before calculating the resulting combination $F_B \circ F_A^{-1}$. In most cases - where there are sufficient participants and but few limited linearity defects in lab data - a simple linear relationship can suffice.

Example of model using linear equation :

F_A : $\qquad y = x \cdot b_A + a_A \qquad \Leftrightarrow \qquad F_A^{-1}: \quad x = y/b_A - a_A/b_A$

F_B : $\qquad z = x \cdot b_B + a_B$

$F_B \circ F_A^{-1}$: $\qquad z = (y/b_A - a_A/b_A) \cdot b_B + a_B$

$\qquad\qquad\qquad z = y \cdot (b_B/b_A) + (a_B - a_A \cdot b_B/b_A)$ (1)

with x the values of the reference lab and y the assigned reference values in Scheme A and z the assigned reference values in Scheme B

Once established equation 1 is used to predict the virtual reference in Scheme B for the samples used in Scheme A (different from those of Scheme B) and the virtual reference values can serve to calculate the virtual scores of laboratories of Scheme A in Scheme B (see Figures 4 and 5).

Where there is no level effect on the bias – i.e. slopes b_A and b_B equal to 1 – the relation (1) simplifies in an addition of a constant term as $z = y + (a_B - a_A)$ as described by O Leray (2008).

Example of application

The model of bridging of Figure 2 can be implemented at the level of national / regional schemes in the frame of multi- lateral comparison for instance to resolve local disputes (Figure 3 on left), or as the way to anchor the national / regional PT schemes to a single central scheme that can provide the commonly accepted truth (Figure 3 on right). This is the latter model proposed and developed by the ICAR through its reference laboratory network. However both models can be used complementarily in dedicated reference systems such as the on-going IDF-ICAR project of Reference System for Somatic Cell Counting.
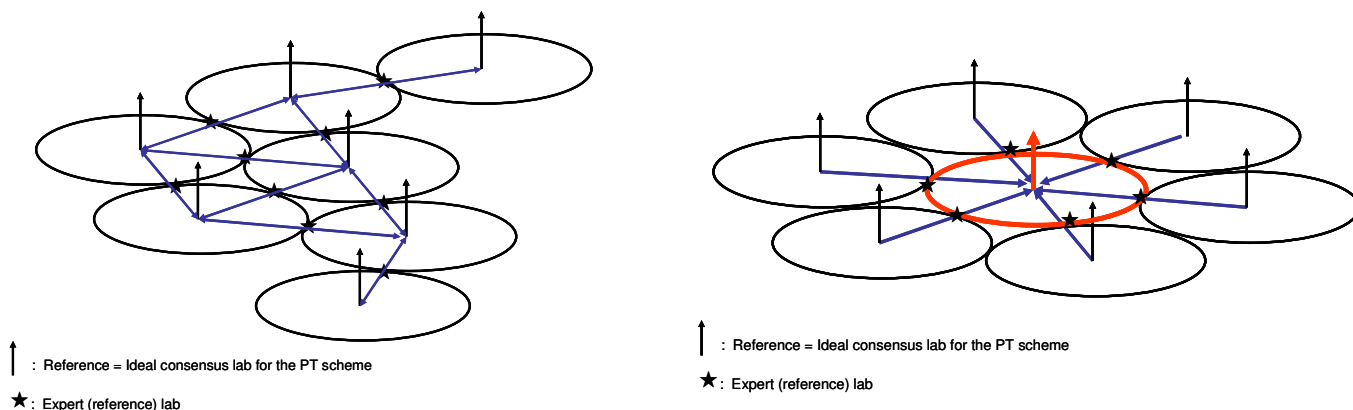
Figure 3. Two-by-two interlinking of PT schemes in a chain or a network (left) ; PT scheme anchorage to a central PT scheme as a model for an international anchorage and traceability (right).

First test application within ICAR

Virtual scoring was made using equation 1 for somatic cell counting in the frame of the ICAR reference laboratory network and PT trial of 2009 (Figure 4). Scores are represented in abscissa as the mean laboratory differences to the reference whereas standard deviation of differences are reported in ordinate (IDF 1999). The reference laboratory of a national PT scheme showed its score (N) reduced in a virtual score close to zero which was then better in line with its score obtained in the international PT trial organised by ICAR. The larger standard deviation of (I) relates to the concentration range of the international significantly higher than in the national PT scheme.
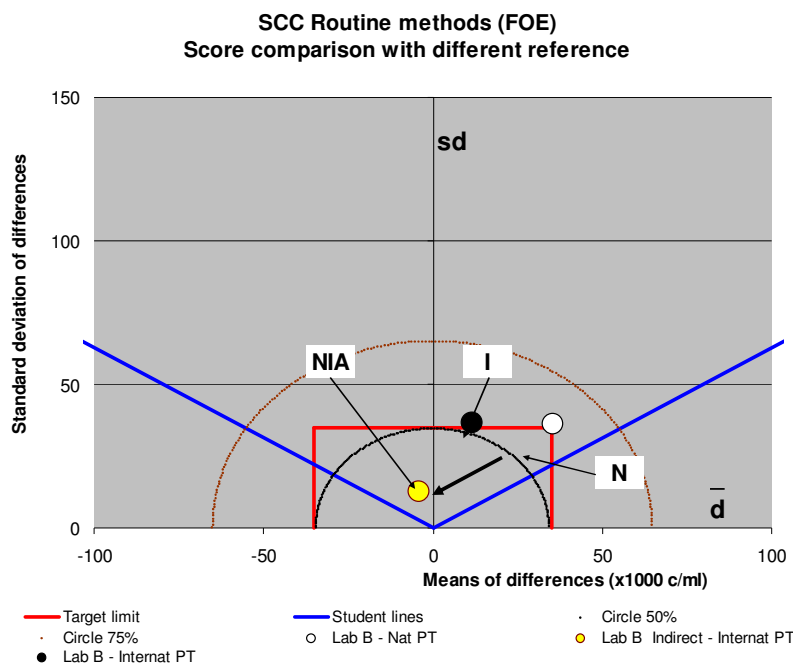
Figure 4. Scores diagram of a reference laboratory in national PT scheme (N), international ICAR PT scheme (I) and national PT scheme internationally aligned (NIA).

Applying the virtual reference to all the laboratories (Figure 5, right) resulted in a shift of the lab population to the left (underestimation) and a reduction of standard deviations in the median part of the diagram. This can be interpreted as a overall slope modification due to either a biased reference or a difference of the reference laboratory performance between national and international schemes. If

confidence is given to the results of the reference laboratory both pictures can comfort each other to indicate adequate diagnosis such as possible troubles with PT samples quality or improper calibration materials reflected in a general negative trend. Other statistics such as the residual standard deviation of regressions and quality control records should then confirm.
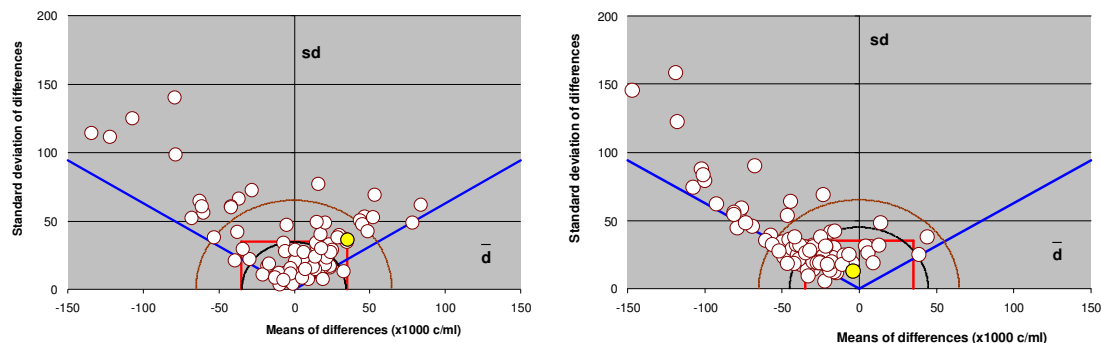
Figure 5. Scores diagrams of participants in national PT scheme : actual as compared to the national reference (left), virtual as compared to the international ICAR reference.

The virtual reference calculation was applied similarly to different PT schemes anchored to ICAR trials in order evaluate the possibility to proceed to a global lab evaluation. Theory was verified on that optimal population centring is obtained onto the average and any national reference not conforming to the international reference would result in larger lab score scattering in the virtual evaluation. Figure 6 illustrates the positions of sub-groups of 20 milk recording laboratories of each scheme before and after national reference alignment onto the international reference of ICAR.
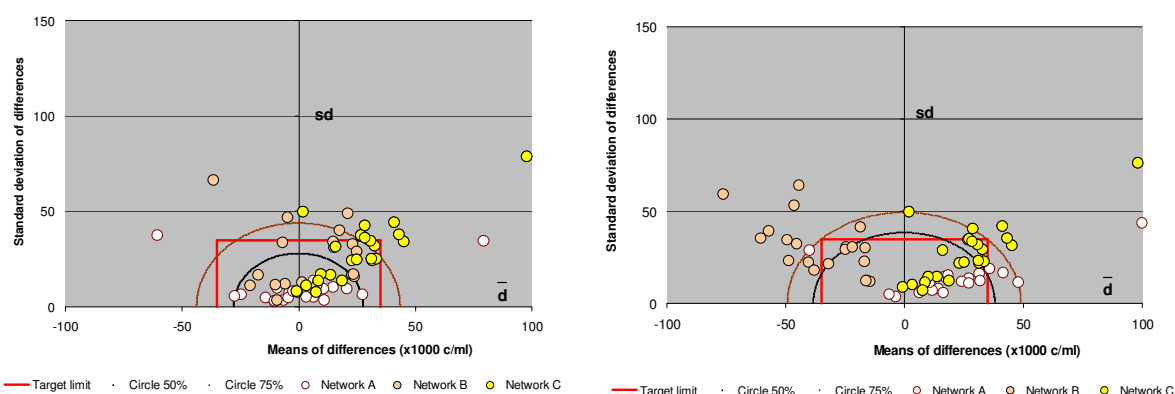
Figure 6. Example of evaluation of laboratories from different national PT scheme : actual as compared to the national reference (left), virtual as compared to the international ICAR reference.

In that example experimental designs (sample types, sample and replicate numbers, concentration ranges) were significantly different between PT schemes thus showing different population pictures with regard to limits stated.

Constraints and requirements

Applying such a system relies on assuring

1- absolute stability of the analytical method used by the reference laboratory during the time gap between the two sample sets testing. This is obtained - either through simultaneous testing in a same test series, subsequently or better by alternating samples of each series, - or linking test run of each sample sets through a significant quality control net with adequate reference materials.

2- the lowest uncertainty of virtual reference estimates through the equation chaining. To achieve this the sample number and the concentration range of the reference PT scheme (Scheme B) should be larger (at least equivalent) to those of the tested PT scheme (Scheme A). The highest correlation of the reference lab with the assigned values of both schemes should be achieved.

Implementing such a system within ICAR would require the former constraints be laid down in appropriate guidelines and a harmonised PT organisation protocol for ICAR be described in line with the ISO 8196 for calibration and ICAR guidelines so as to make PT scheme comparables with regard to performance evaluation.

Conclusion

The interconnection of PT schemes and international anchorage are technically possible for every type of methods thanks to the principle here above developed. The PT scheme interlinking system enables comparing different PT schemes and laboratories of different PT schemes and move forward to harmonisation. It enables to assess national PT schemes through international anchorage to the reference PT schemes organised for the ICAR reference laboratory networks.

Users' awareness is needed on particular cautions necessary to be taken with regard to proper virtual reference estimation and that, all in all, fair comparisons between independent PT schemes can only result from the harmonisation of PT organisation protocols within ICAR.

List of References

Baumgartner, C., van den Bijgaart, H. 2010. International reference system for somatic cell counting in milk - A world wide challenge. Proceedings of the 37th ICAR Biennial Session, Riga (Latvia), 31 May-4 June 2010. ICAR technical Series No.14. 271-276.

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Leray, O., 2008. ICAR AQA strategy – International anchorage and harmonisation. Fourth ICAR Reference Laboratory Network Meeting. Proceedings of the 36th ICAR Biennial Session, Niagara Falls (USA), 16-20 June 2008. ICAR technical Series No.13. 295-300.

Leray, O., 2008. Interlaboratory reference systems and centralised calibration – Prerequisites and standard procedures. Fourth ICAR Reference Laboratory Network Meeting. Proceedings of the 36th ICAR Biennial Session, Niagara Falls (USA), 16-20 June 2008. ICAR technical Series No.13. 301-305.

MA SC

***ICAR Sub-Commitee on
Milk Analysis***

Proficiency Testing scheme interlinkage
-
International laboratory anchorage

Olivier Leray, 39800 Poligny, France.

Meeting of ICAR Reference Laboratory Network, 28 May - 38th ICAR Biennial Session,   1
Cork (Ireland)

Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

3- Choice for an universal reference

4- Linking PT schemes

5- Different levels of implementation

6- Application to somatic cell counting

7- Conclusion

2

The reference in the ICAR AQA System

☞ **Objective :**

- Establish the **true content** of component in DHI milk

- ICAR Guidelines :
  - a- methods internationally standardized
  - b- standardized methods tightly fitted / anchored to a-

- Ref methods slow => **calibration of routine methods** with reference

☞ **2 ways to calibrate routine methods :**

- In-house calibration :   individual laboratory

- Centralized calibration :   interlaboratory study

☞ **Secure DHI analysis thr. Quality Control :**

- Internal (QC, SRMs) ;   **external (CRMs, PTs)**

- **Reference** and routine methods

Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

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4

What is / where is the « analytical truth » ?

« Truth » stated by **reference methods** but …

is a **relative concept**

⇩

Reference methods provide results with **uncertainty !**

⇩

Precision characteristics (ISO 5725) :
repeatability, r=2.8 sr ; reproducibility, R=2.8 sR,

⇒ *Between lab variation sL² = sR² - sr²*

⇩

Deviations within limits are permitted :
> *max range between 2 laboratories L = 2.8 sL*
> *max lab bias from the reference +/-2 sL*

What is / where is the « analytical truth » ?

Component	ISO	sR	sr	sL	R	r	L	+/-2 sL
Fat g/100g	1211	0,02	0,014	0,014	0,056	0,039	0,040	0,029
Protein g/100g	8968	0,018	0,014	0,011	0,050	0,039	0,032	0,023
SCC(10³ c/ml)	13366-2	45	20	40	126	56	113	81
SCC rel (750.10³ c/ml)	13366-2	6%	3%	5%	17%	8%	15%	10%

Ranges allowed between two laboratories chosen at random

Lab bias limits allowed from the reference for « normal laboratories »

⇒ **Reduction thr. AVERAGING ⇒ laboratory network MEANS as reference**

Every national network defines its own reference
None knows whether it differs from the others
… nor how far !

ICAR INTERNATIONAL REFERENCE LABORATORY NETWORK

A = B = C = D = E = …?
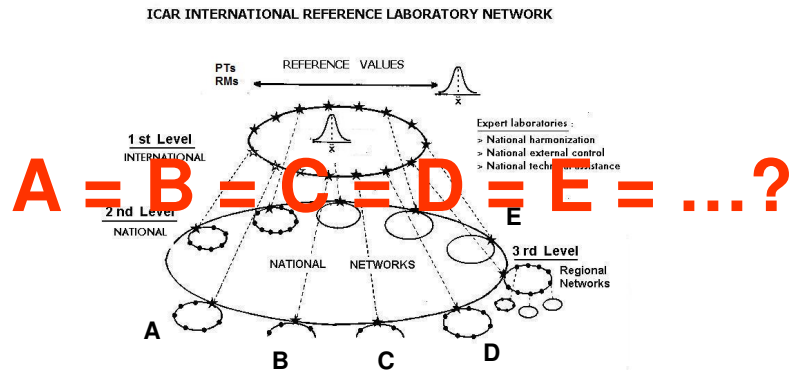
Assumption that the group gives the true reference is relative

Different conditions of interlaboratory studies (lab number, PT design and protocol, methods involved) **can influence population results** and the resulting **references**.

7

Recalls on calibration and PT Statistics

Statistical parameters to evaluate lab trueness performances and calibration

8

ICAR Interlaboratory Proficiency Study - March 2008

2 Labos OUT OF THE TARGET (10 %)

Trial of : 03/03/2008

Sd

12
20

225

21 laboratories
10 samples

150

Student 5 %

Student 5 %

Statistical parameters to evaluate lab trueness performances and calibration

75

D

$\overline{d}$

sd

0

-150   -75   —   0   75   150

Target limits : d = +/- 35.10³ cells / ml of milk

Sd =   35.10³ cells / ml of milk

9

PT study

Protein by Kjeldahl

ICAR RLN

sd

66 participants
10 samples in duplicates

$\overline{d}$ = mean bias
units : g/kg

-0.50   0.00   0.50

Foreign labs   **Country labs**

> 0.010 % protein

10

PT scheme interlinkage and international laboratory anchorage

PT study

Somatic Cell Counting

70 participants
10 samples in duplicates   sd

Fluoro-opto-electronic methods

$\overline{d}$ = mean bias
units : g/kg

-50   0   50

ICAR RLN   **Foreign labs**   **Country labs**

35 10³   25 10³

5%   3%

11

Diversity in PT scheme in Europe => different references ?

Scheme A   **Scheme B**   **Scheme C**
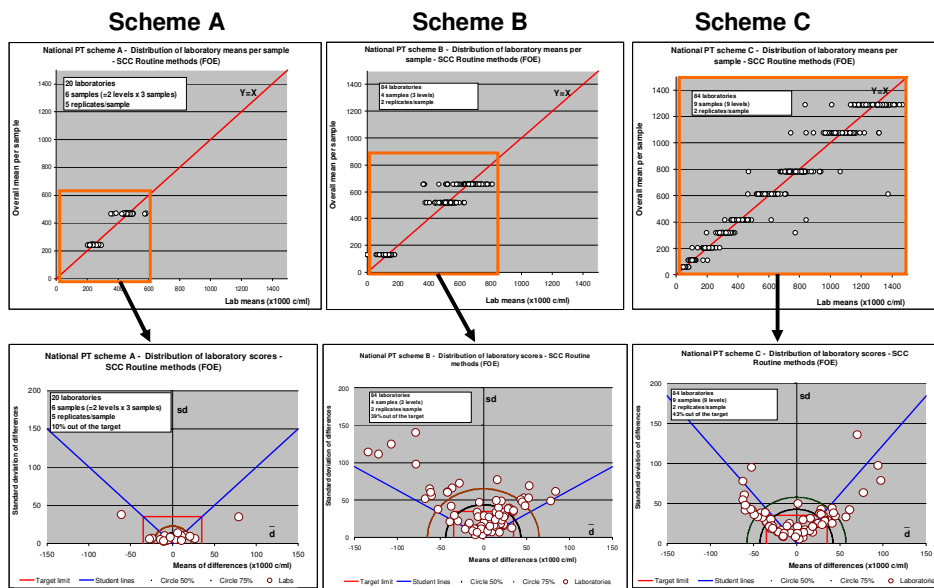
12

Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

3- Choice for an universal reference

4- Linking PT schemes

5- Different levels of implementation

6- Application to somatic cell counting

7- Conclusion

13

ICAR INTERNATIONAL REFERENCE LABORATORY NETWORK

14

Truth = agreement of all the stakeholders to reach common goals

i.e. worldwide harmonisation for ICAR

⇩

ICAR choice = International network of reference labs using reference methods

⇒ **International recognition**   **representativeness (political),**

⇒ **Highest competence & training**

   suitable methodology & capability (technical)

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Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

3- Choice for an universal reference

4- Linking PT schemes

5- Different levels of implementation

6- Application to somatic cell counting

7- Conclusion

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Principle of interlinking schemes with expert laboratories

O. Leray, ICAR Session, Niagara Falls, 2008 ; IDF | ICAR – SCC RS, 2010

Reference A   ☆
                Reference lab   **Reference B**

$$F_B \circ F_A^{-1}$$

$$F_A \qquad F_B$$

Lab x

?

PT scheme A   **PT scheme B**

——————— Existing routes

– – – – – Unknown direct routes

To each route there is a specific mathematical relationship for linking

16

Inter-linking relation using linear equations

O. Leray, 2010 – IDF | ICAR – SCC RS project (underway)

$$F_A : \quad y = x \cdot b_A + a_A \qquad \Leftrightarrow \qquad F_A^{-1}: \quad x = y/b_A - a_A/b_A$$

$$F_B : \quad z = x \cdot b_B + a_B$$

y = Real reference of scheme A

z = Virtual reference of scheme A in scheme B

$$F_A^{-1} \circ F_B \qquad z = (y/b_A - a_A/b_A) \cdot b_B + a_B$$

$$z = y \cdot (b_B/b_A) + (a_B - a_A \cdot b_B/b_A)$$

No level effect => z = y + (a_B - a_A) = y + ($\overline{d}_A$ - $\overline{d}_B$)

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Interlinkage of national PT schemes <=> PT scheme networking

O. Leray, 2010 – IDF | ICAR – SCC RS project (underway)

Comparison & 2-by-2 alignment

With no knowledge on where is real « Truth »

19

↑ : Reference = Ideal consensus lab for the PT scheme

★ : Expert (reference) lab

Anchorage to an international reference scheme

O. Leray, 2010 – IDF | ICAR – SCC RS project (underway)
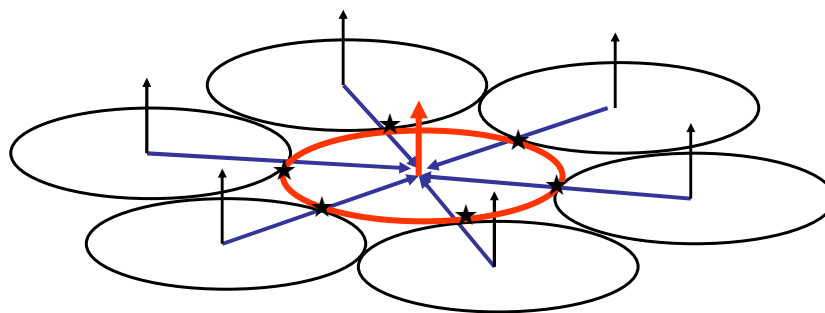
Alignment to a real « Truth » stated by consensus & comparison

20

↑ : Reference = Ideal consensus lab for the PT scheme

★ : Expert (reference) lab

Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

3- Choice for an universal reference

4- Linking PT schemes

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7- Conclusion

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SCC International PT scheme : Levels and results
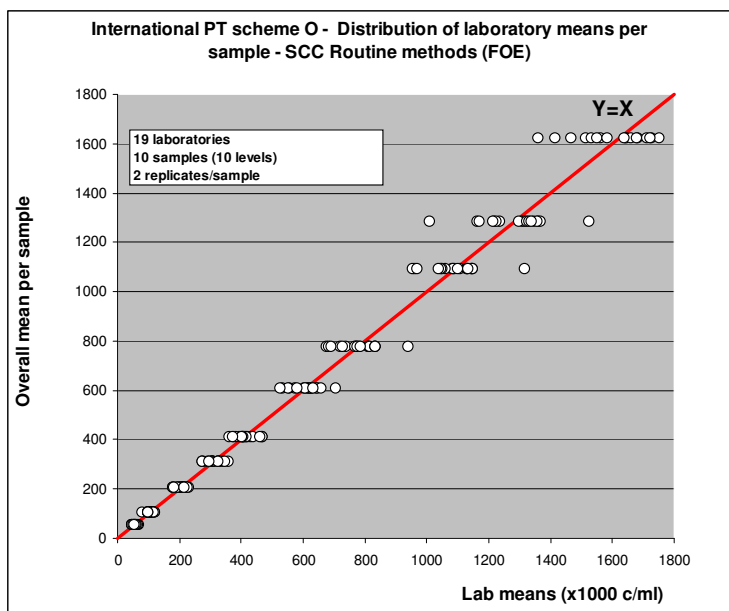
ICAR PT scheme Sept. 2009

22

International PT scheme : Reference Laboratory scores

International PT scheme O - Distribution of laboratory scores - SCC Routine methods (FOE)

19 laboratories
10 samples (10 levels)
2 replicates/sample
59% out of the target

sd

$\overline{d}$

Means of differences (x1000 c/ml)

Target limit · Student lines · Circle 50% · Circle 75%
○ Laboratories · ● Lab A · ● Lab B · ● Lab C

ICAR PT scheme Sept. 2009

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International PT scheme anchorage

ICAR PT scheme Sept. 2009

Scheme O

International level ⇨

National / regional level
⇩

Scheme A

Scheme B

Scheme C
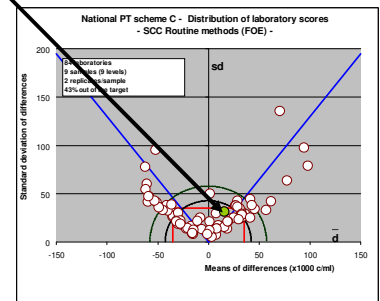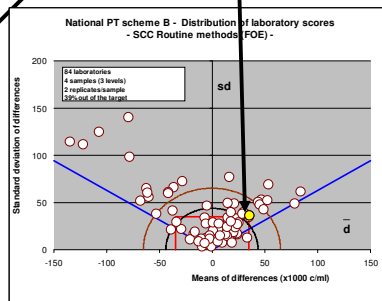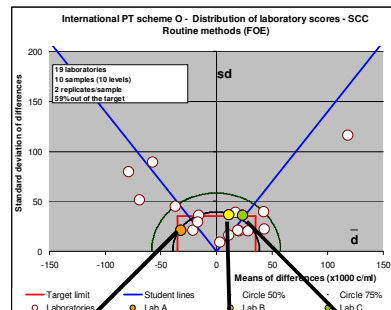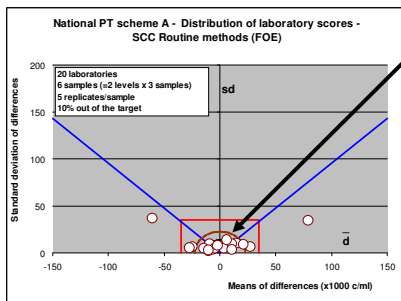
24

Alignment of reference for a national scheme B onto an international scheme :

Different scores of Ref Lab B

25
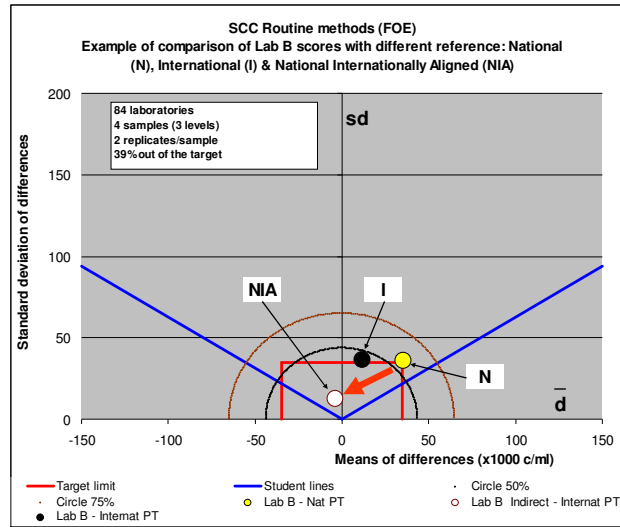
PT scheme interlinking of a national scheme B to an international scheme O

=> Evolution of labs scores of scheme B

National PT (actual)   **National PT Internat^ly Aligned (virtual)**
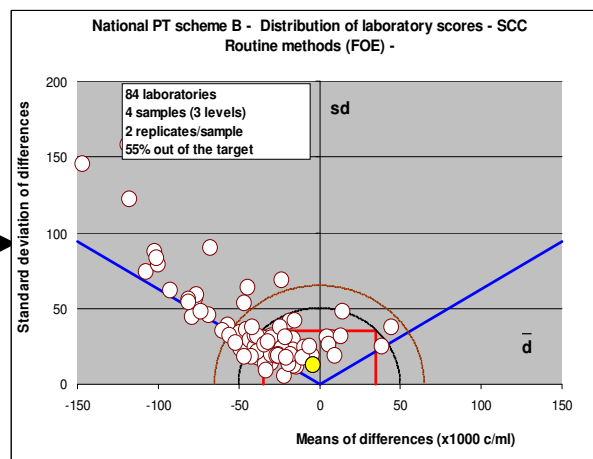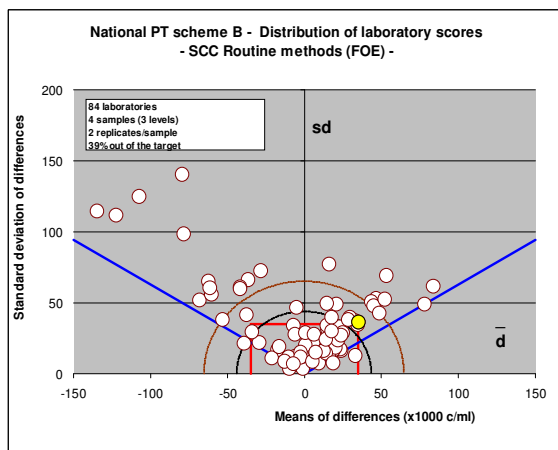
Local PT studies can show only a part of the problem if there si no external input (Reference PT or RMs) to « give the Truth »

26

Different locations of Lab B in 4 different schemes A, B, C & O

Ref Lab and/or references questionable ?
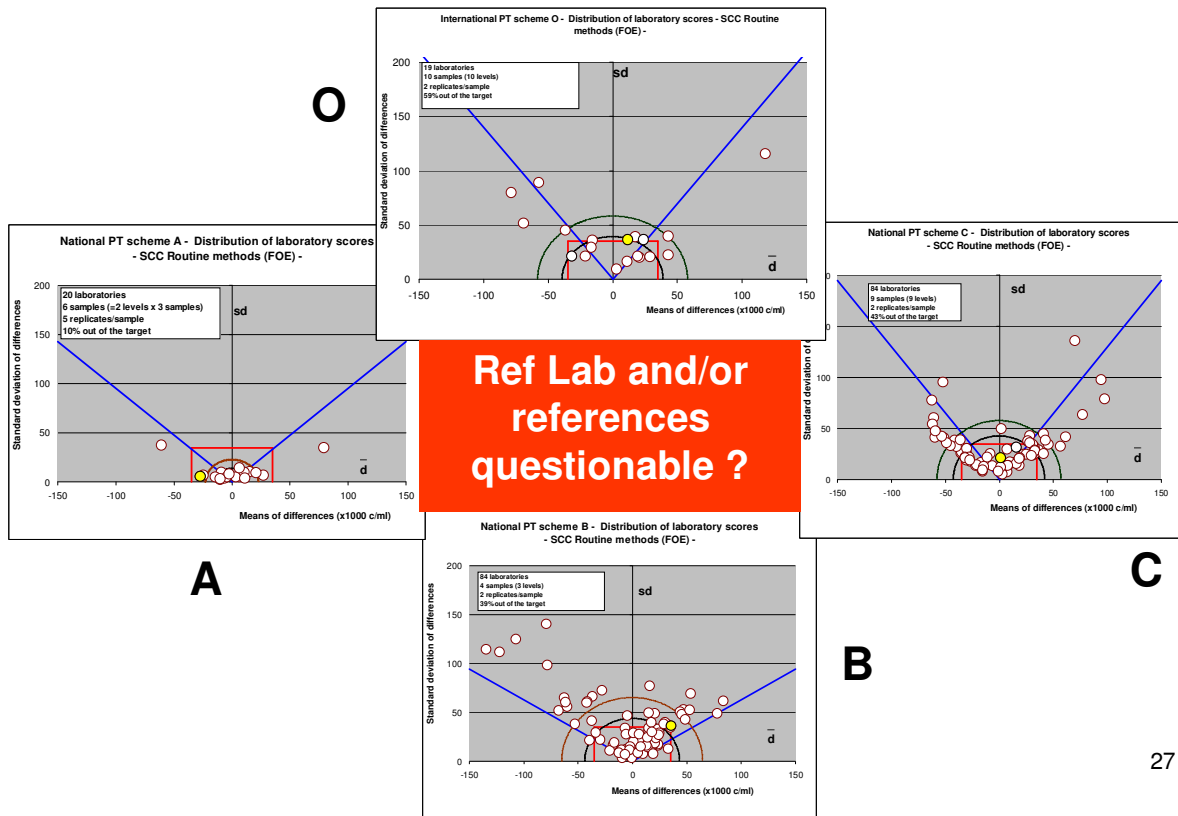
Simultaneous comparison of DHI labs of three countries

3 National PTs in SCC
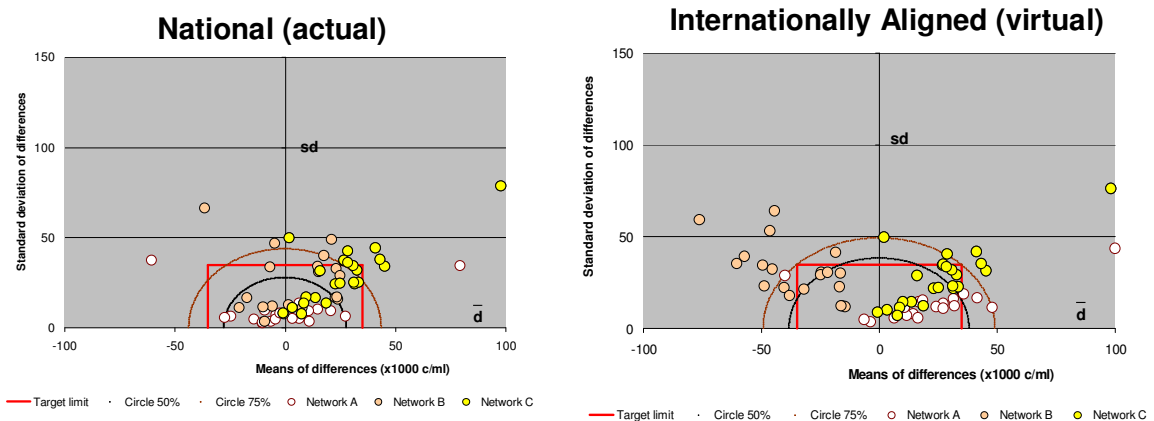
Labs score scattering would stay unchanged if Ref Lab B would have same scores (position) in harmonized PT conditions !

Ref Lab and/or references questionable ?

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R A N K I N G   L A B O R A T O R I E S

National (actual)

Rank	Rank%	ID	d	sd	D
1	2%	A6	3,4	4,9	6,0
2	3%	A11	-4,5	4,9	6,6
3	5%	A7	-6,4	3,6	7,4
4	6%	B8	-0,7	7,6	7,7
5	8%	C84	-0,9	8,0	8,0
6	9%	A3	6,3	5,0	8,0
7	11%	A1	-3,9	7,1	8,1
8	13%	A19	-2,3	8,1	8,5
9	14%	B9	-9,3	3,2	9,9
10	16%	A2	-10,1	2,4	10,4
11	17%	C21	7,4	7,6	10,6
12	19%	A17	-10,2	3,1	10,6
13	20%	C22	3,2	11,1	11,5
14	22%	A14	11,0	3,4	11,5
15	23%	B21	1,6	12,6	12,7
16	25%	B2	-5,9	12,0	13,4
17	27%	A9	-9,1	9,8	13,4
18	28%	B22	7,7	11,0	13,4
19	30%	A13	10,8	9,6	14,4
20	31%	A10	-14,3	4,7	15,0
21	33%	A15	6,4	13,8	15,2
22	34%	B18	-9,9	11,7	15,3
23	36%	C14	8,6	13,8	16,2
24	38%	A4	15,0	10,2	18,1
25	39%	C13	9,7	17,0	19,6
26	41%	C2	13,6	16,5	21,4
27	42%	A16	20,8	9,2	22,8
28	44%	C15	18,6	13,6	23,0
29	45%	B13	-20,8	11,0	23,5
30	47%	B4	-17,4	16,8	24,2
31	48%	A8	-24,5	6,5	25,4
32	50%	A20	-27,2	5,7	27,8
33	52%	A5	27,3	6,4	28,0
34	53%	B6	22,8	16,6	28,2
35	55%	B19	23,8	15,9	28,6
36	56%	B24	23,6	17,2	29,2
37	58%	C8	22,9	24,2	33,3
38	59%	B7	15,1	30,8	34,3
39	61%	B17	-6,9	33,7	34,4
40	63%	C19	24,9	24,8	35,1
41	64%	C25	16,0	31,3	35,2
42	66%	B11	14,8	33,8	36,9
43	67%	B23	25,1	28,7	38,1
44	69%	C1	31,5	24,0	39,6
45	70%	C17	31,1	25,3	40,1
46	72%	B3	23,1	32,8	40,1
47	73%	C12	33,3	25,2	41,8
48	75%	B15	17,7	39,9	43,6
49	77%	C10	32,6	31,7	45,5
50	78%	C6	26,6	37,1	45,7
51	80%	C20	28,5	36,1	46,0
52	81%	C18	26,9	37,4	46,0
53	83%	C16	30,8	34,3	46,1
54	84%	B5	-4,7	46,7	46,9
55	86%	C7	1,9	49,8	49,8
56	88%	C5	28,5	42,6	51,3
57	89%	B16	20,9	48,7	53,0
58	91%	C9	45,3	34,0	56,6
59	92%	C23	43,0	38,0	57,4
60	94%	C3	41,0	44,2	60,3
61	95%	A18	-60,6	37,3	71,2
62	97%	B14	-36,6	66,3	75,7
63	98%	A12	79,3	34,6	86,5
64	100%	C11	98,0	78,5	125,5

International aligned(virtual)

Rank	Rank%	ID	d	sd	D
1	2%	A8	-3,7	3,4	5,1
2	3%	A20	-6,4	4,6	7,9
3	5%	A10	6,5	5,6	8,6
4	6%	C84	-0,7	8,7	8,7
5	8%	C22	3,4	9,9	10,4
6	9%	C21	7,6	7,2	10,5
7	11%	A9	11,7	7,0	13,6
8	13%	C14	8,8	11,5	14,5
9	14%	A2	10,7	11,0	15,3
10	16%	A17	10,6	11,3	15,5
11	17%	A7	14,4	7,8	16,3
12	19%	A11	16,3	5,8	17,3
13	20%	C13	9,9	14,6	17,6
14	22%	B23	-14,4	11,8	18,6
15	23%	C2	13,9	14,2	19,8
16	25%	B19	-15,7	12,2	19,9
17	27%	B24	-15,9	12,1	20,0
18	28%	A1	16,9	14,5	22,3
19	30%	C15	18,8	12,1	22,4
20	31%	A19	18,5	15,0	23,8
21	33%	A6	24,2	11,7	26,9
22	34%	B6	-16,7	22,7	28,2
23	36%	A15	27,2	11,1	29,4
24	38%	A3	27,1	13,3	30,2
25	39%	C8	23,1	21,7	31,7
26	41%	C25	16,2	28,8	33,0
27	42%	C19	25,1	22,1	33,5
28	44%	A14	31,9	12,0	34,1
29	45%	B3	-16,4	29,9	34,1
30	47%	A13	31,7	15,9	35,5
31	48%	B15	-21,8	30,6	37,6
32	50%	B11	-24,7	29,1	38,2
33	52%	B22	-31,8	21,3	38,3
34	53%	C1	31,7	21,5	38,3
35	55%	C17	31,3	23,1	38,9
36	56%	B7	-24,4	30,3	38,9
37	58%	A4	35,8	18,8	40,4
38	59%	C12	33,5	22,7	40,5
39	61%	B21	-37,9	17,6	41,8
40	63%	C6	26,8	34,5	43,7
41	64%	C10	32,9	29,1	43,9
42	66%	C20	28,7	33,4	44,1
43	67%	C18	27,1	35,0	44,2
44	69%	C16	31,0	31,8	44,4
45	70%	A16	41,6	16,4	44,7
46	72%	B16	-18,6	41,3	45,3
47	73%	B8	-40,2	22,3	46,0
48	75%	A18	-39,8	28,8	49,1
49	77%	A5	48,1	11,4	49,4
50	78%	C5	28,7	40,3	49,5
51	80%	C7	2,1	49,5	49,6
52	81%	B9	-48,8	23,2	54,1
53	83%	C9	45,5	31,4	55,3
54	84%	B2	-45,4	32,3	55,7
55	86%	C23	43,2	35,4	55,9
56	88%	C3	41,2	41,8	58,7
57	89%	B18	-49,4	34,2	60,1
58	91%	B4	-56,9	39,0	69,0
59	92%	B13	-60,3	35,0	69,7
60	94%	B17	-46,4	53,2	70,6
61	95%	B5	-44,2	63,9	77,7
62	97%	B14	-76,1	58,9	96,2
63	98%	A12	100,1	43,7	109,2
64	100%	C11	98,2	76,0	124,1

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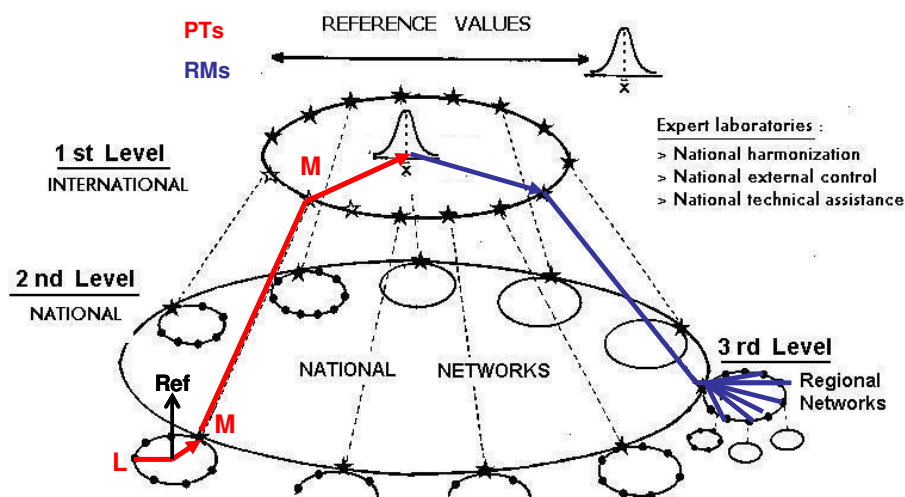
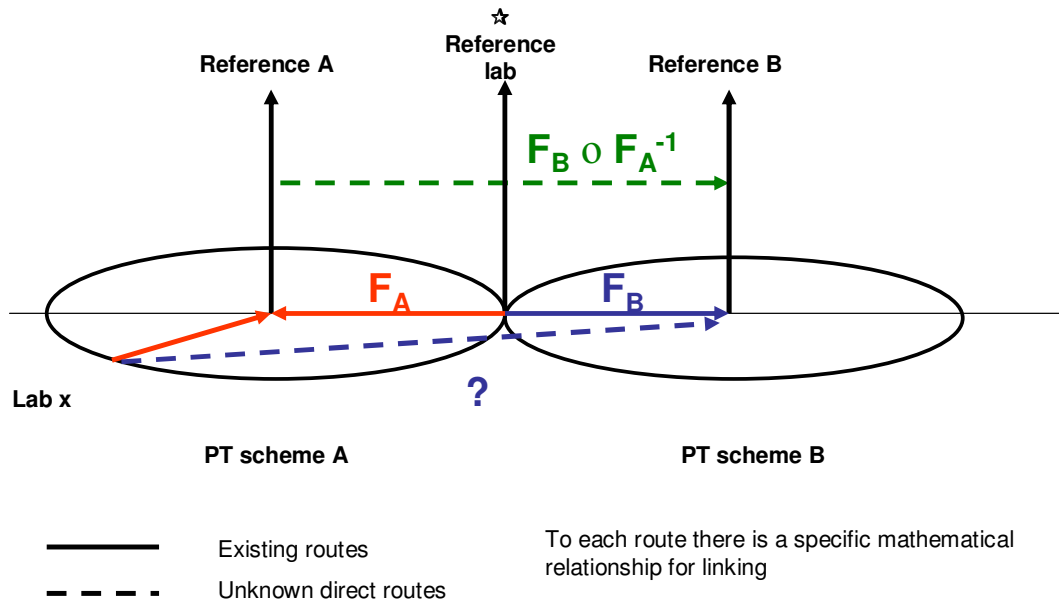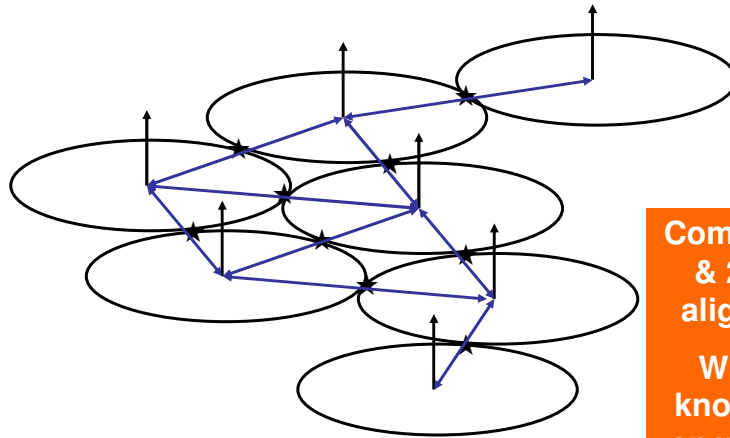# Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

3- Choice for an universal reference

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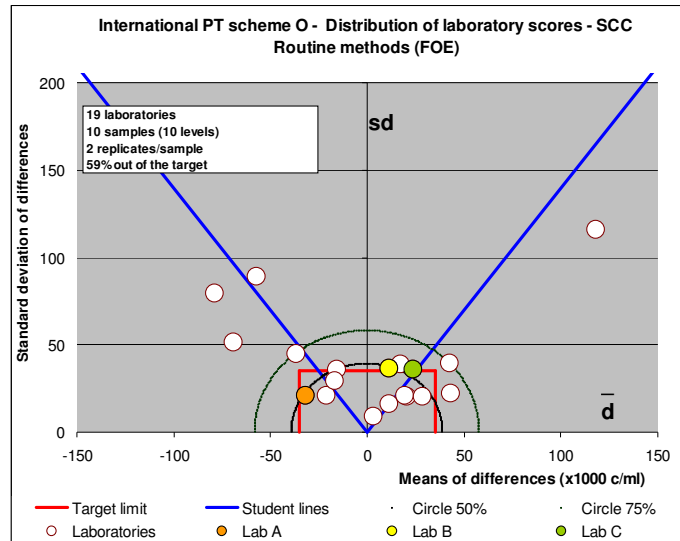
6- Application to somatic cell counting

7- Conclusion

30

Conclusion

Pre-requisites for international lab anchorage and intercomparison

1- An international network of national expert laboratories (e.g. ICAR Reference Laboratory Network)

2- Ref labs acquiring high skill and competence :

$\Rightarrow$ High stability in performance : constant low bias & low repeatability.

$\Rightarrow$ Tight anchorage thr. a net of RMs and PTs (QC)

3- Simultaneous / close participation of expert labs in international and national PT schemes.

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Anchoring labs to an international reference and comparing them on a unique scale is possible

ψ Existing PT schemes are appropriate tools

ψ PT schemes work independently with applying different protocols:

$\Rightarrow$ To **establish new ICAR guidelines** to harmonise PT protocols thereby enable comparable and fair lab performance evaluation

ψ Preliminary cautions of simultaneousness, analytical stability (QC), high ref lab performance to be achieved:

$\Rightarrow$ To **establish new ICAR guidelines** to assure trusty correspondence between PT schemes.

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ICAR Inter Laboratory Proficiency Testing – Ireland Update - *Review 2002 - 2011*

Martin Burke [1] , Des Eason [2], Anne-Marie McAuliffe [2]

[1] ICBF, Highfield House, Shinagh, Bandon (Cork), Ireland

[2] Technical Services Laboratory. Food Research Centre,Teagasc, Moorepark, Fermoy (Cork), Ireland

Irish Milk Recording Laboratories & ICAR

ICAR Inter Laboratory Proficiency Testing – Ireland Update

Review 2002 - 2011

Martin Burke (ICBF), Des Eason (Teagasc),
Anne Marie McAuliffe (Teagasc)

May 28th ICAR 2012 Cork

Overview Ireland : Prof. Test Structure

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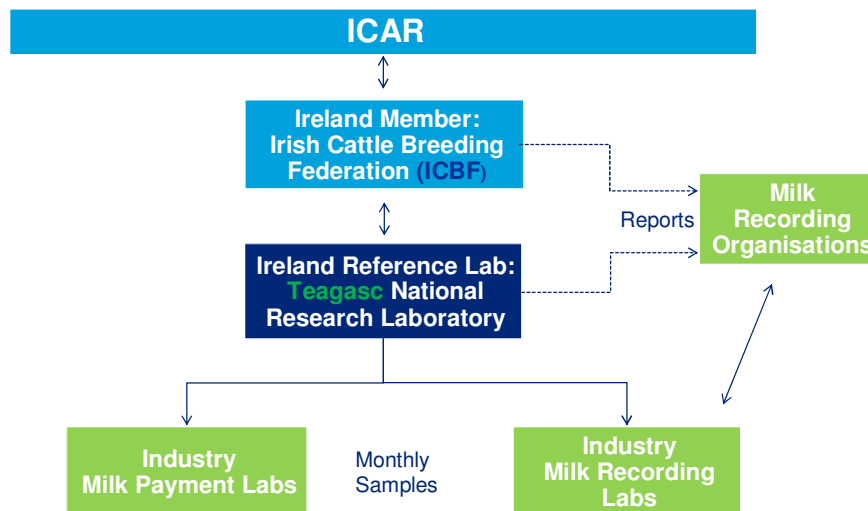
10 Yrs Ago - Ireland Milk Recording Labs 2002

**2002 MILK RECORDING LABS
380K cows recorded
- 8 laboratories !**

Milk recording only

Milk recording & payment

Source: ICBF/IFA
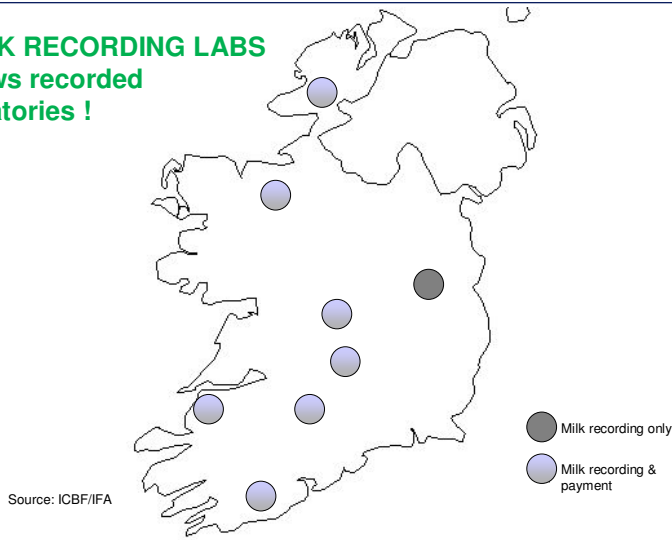
3

Current - Ireland Milk Recording Landscape 2012

**2012 - MILK RECORDING LABS
4 laboratories and > 500k cows
recording**

Tipperary Co-op
Recording ~ 14k cows

**Munster Milk
Recording**
~ 290k cows

**Progressive Genetics/
NML**
Milk Recording
~ 210k cows

Milk recording only

Milk recording & payment

Source: ICBF/IFA
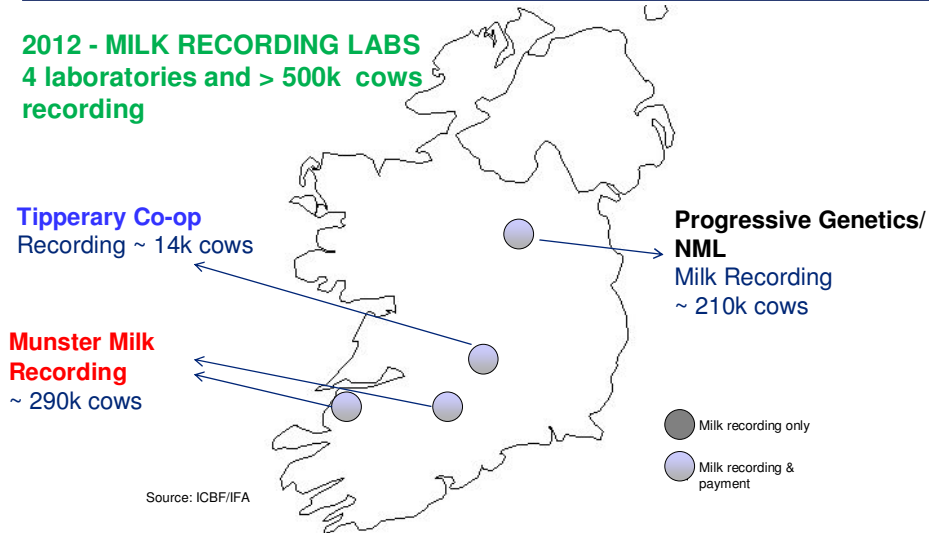
4

Moorepark International Ring Test – ICAR Labs

❑ Moorepark participates in ICAR International ring tests twice a year

❑ For each test 10 milks are done by Moorepark for

% Fat   +/- 0.020 Mean diff and Std Dev of 0.030
% Protein   +/- 0.025 Mean diff and Std Dev of 0.020
% Lactose   +/- 0.100 Mean diff and Std Dev of 0.100
Somatics   +/- 35.1 Mean diff and Std Dev of 35.1 (cells by 1000)

Due to Staff retirements and new hire delay, the International Ring test was deferred – resuming again in 2012. !

5

Monthly Inter Lab Scoring System

❑ Whether Milk Rec. or Milk Payments - participate in Monthly ring tests.

❑ Teagasc Moorepark (National Dairy Research Lab) are contracted by ICBF to conduct the proficiency ring test each year. The system is very simple

❑ Each month each lab receives 2 samples to test from Teagasc Moorepark

❑ Test results to Moorepark & compared vs Reference Methods *.

Tolerances; Fat +/-0.10, Prot +/-0.07, Lact +/-0.10, SCC +/- 15%

1 Penalty Point and a Yellow Card if Difference is > tolerance

2 Penalty Points and a Red Card if Difference is > 2 x tolerance

(* Rose Gottlieb (Fat), Kjeldahl (Prot) Polarimeter (Lact), SCC CECELAIT SRMs used)

6

Monthly Inter Lab Scoring Results 2011

Moorepark Proficiency Programme Report 2011

Number of Yellow & Red cards received by each lab in the year 2011

Lab Code	Fat Y	Fat R	Fat No of Tests	Protein Y	Protein R	Protein No of Tests	Lactose Y	Lactose R	Lactose No of Tests	SCC Y	SCC R	SCC No of Tests	TOTAL Y	TOTAL R	No of Tests
E	1	0	14	0	0	14	0	0	12	5	0	13	6	0	59
J	2	0	12	0	0	12	0	0	12	0	0	12	2	0	50
K	1	0	18	2	1	18	0	0	16	0	0	17	3	1	73
S	0	0	10	0	0	10	0	0	10	1	0	9	1	0	40
Total	**4**	**0**	**54**	**2**	**1**	**54**	**0**	**0**	**50**	**6**	**0**	**51**	**12**	**1**	**222**

Y 5.4%, **R** 0.5%

7

Monthly Inter Lab Scoring Results 2002 -2011

Moorepark Proficiency Programme Summary 2002 > 2011

	Fat Y	Fat R	No of Tests	Protein Y	Protein R	No of Tests	Lactose Y	Lactose R	No of Tests	SCC Y	SCC R	No of Tests	TOTAL Y	TOTAL R	No of Tests
2011 Totals	4	0	54	2	1	54	0	0	50	6	0	51	12	1	222

Percentages over 10 year period

	% Y	% R		% Y	% R		% Y	% R		% Y	% R		% Y	% R	
% in 2011	7.4	0.0	☺	3.7	0.5	☺	0.0	0	☺	11.8	0	☺	5.4	0.5	☺
% in 2010	12.6	2.8		8.2	0.9		3.1	0		15.3	3.0		8.6	1.5	
% in 2009	11.9	0.0		7.7	0.3		3.2	0.3		14.4	2.3		8.3	0.6	
% in 2008	13.1	3.4		8.0	0.6		3.9	0.9		8.4	1.9		7.6	1.5	
% in 2007	14.7	1.7		6.1	0.9		3.7	0.3		7.0	1.1		8.0	1.0	
% in 2006	13.0	1.2		7.8	0.0		0.7	0.0		11.7	1.2		8.3	0.6	
% in 2005	14.7	2.7		9.5	0.8		5.2	0.0		7.4	1.0		9.4	1.2	
% in 2004	13.4	2.5		9.9	0.5		6.3	0.0		15.4	4.8		11.0	1.8	
% in 2003	12.0	2.2		6.1	0.5		5.2	0.5		5.8	1.6		4.7	1.6	
% in 2002	9.2	1.2		9	0.6		4.7	0		11.7	2		8.6	0.9	
Averages	*12.2*	*1.8*		*7.6*	*0.6*		*3.6*	*0.2*		*10.9*	*1.9*		*8.0*	*1.1*	

8

Inter Lab Results & Milk Recording Managment

2002 – 8 Milk recording agencies, ageing equip = difficult to review/action results

2012 – 2 Milk Recording agencies account for 97% of cows recorded

Higher volume, newer equip

Easier to review with 2 x Milk Recording Management teams

9

Inter Lab Results & Milk Recording Managment

2012 – 2017 Future challenges/Opportunities Irish Labs

- Optimir (in progress)

- More from sample - Carry Over (sampling and lab?)

- In Line analysers (how close?…)

end

10

International Strategies and New Developments

in Milk Analysis

Part 2 – New analytical challenges for milk recording

"International Strategies and New Developments in Milk Analysis"

Part 2: New analytical challenges for milk recording

16.45-17.10: **Determination of protein composition in milk by mid-infrared spectrometry**
Marion Ferrand (Institut de l'Elevage, FR)

17.10-17.35: **Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability,**
Martino Cassandro & Massimo De Marchi (Univ. of Padova, IT)

17.35-18.00: **Merging of spectral datasets from different MIR instruments used in the routine analysis of milk,**
Juan Antonio Fernández (CRA-W, Gembloux, BE)

18.00-18.30: **Discussion**

18.30: Closure of the meeting.

Determination of protein composition in milk by mid-infrared spectrometry

M. Ferrand[1], G.Miranda[2], H. Larroque[3], O. Leray[4], S. Guisnel[1], F. Lahalle[1, 5], M. Brochard[1], P.Martin[2]

[1] Institut de l'Elevage, 149 rue de Bercy, 75595 Paris cedex 12, France, marion.ferrand@idele.fr
[2] INRA, UMR1313, Animal Genetics and Integrative Biology, Domaine de Vilvert 78352 Jouy-en-Josas cedex, France
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Abstract

The fine milk composition is currently studied with attention, some components being of a particular interest. To get a better description of these components, it is necessary to have rapid and reliable methods to analyze several hundreds of milk samples in order to estimate genetic and environmental effects on milk quality.

An innovative method based on liquid chromatography coupled with mass spectrometry was developed to identify and quantify the main milk proteins and some works are now on-going to determine milk protein composition from MIR (Mid Infra-Red) spectra usually obtained by milk analysing laboratories.

The first results show that it is possible to estimate each of the 4 casein contents with a good accuracy, especially in ewe's and cow's milk. By contrast, it seems more difficult to estimate the whey proteins contents. Some improvements are under evaluation to enhance the performance of these equations.

Keywords: milk proteins, liquid chromatography, mass spectrometry, mid infrared spectroscopy

Introduction

Milk is a complex product which contains a lot of components with different properties. The main milk proteins are divided into two important families: caseins (α_{s1}, α_{s2}, β, κ) accounting for 80% of milk proteins, and whey proteins (including α-lactalbumin and β-lactoglobulin) which represent *ca.* 20%, in cattle. Some of these proteins are particularly important for cheese production, for example a milk with a high content in κ-casein will clot rapidly and provide a firm curd (Grosclaude, 1988).

A part of the PhénoFinLait programme consists in developing two methods to analyse the protein composition of milk: the first one is a qualitative and quantitative determination using liquid chromatography coupled with mass spectrometry (LC/MS), the second one aims to estimate some major milk proteins contents from MIR spectral data. The LC/MS reference method allows quantification and characterization of the six main proteins, but this method is time consuming and more expensive than the MIR spectrometry that is used in routine in control laboratories. Previous studies (De Marchi, 2009 – Rutten, 2011 – Bonfatti, 2011) show that it was possible to estimate milk protein composition using this technology, but the errors remained high, in particular as far as κ-casein and the two main whey proteins are concerned.

Materials and methods

MIR spectra database

A MIR spectra database has been created between 2009 and 2011 with 73 149 cow milks from 1 072 herds, 33 623 ewe milks from 167 herds and 54 932 goat milks from 191 herds distributed in different areas of France. Spectra were recorded on Foss FT6000 or FT+ and Bentley spectrometers.

First, studies on proteins were focused on Foss data. Some spectra areas were removed following the constructor recommendations (Foss, 1998) since these areas are sensitive to water molecule. Finally, we kept wavelengths from 965 to 1544 cm^{-1}, from 1716 to 2272 cm^{-1} and from 2434 to 2970 cm^{-1}

Quantitative and qualitative analysis using LC/MS

Concomitantly, milks from 271 cows of different breeds (Montbéliarde, Holstein and Normande) 157 ewes (Lacaune and Manech-Tête-Rousse), 151 goats (Saanen and Alpine) were selected and analyzed with an innovative methodology based on Liquid Chromatography (Ultimate 3000 HPLC from Dionex) coupled with Mass Spectrometry (MicroToF focus from Bruker). This method (Miranda, Bianchi and Martin, manuscript in preparation) allows the identification and a relative quantification of the 6 main milk proteins: $\square$-casein (glycosylated or not), α_{s1}, α_{s2} and β-caseins, as well as β-lactoglobulin and α-lactalbumin. The quantification is based on the integration of the UV signal recorded at 214 nm of each peak of the chromatogram. Surfaces are expressed as percent of the total of peaks of the chromatogram. The identification is achieved by comparing the observed masses of each protein to the predicted ones referenced in a milk protein masses database designed and implemented by the authors (APP: IDDN.FR.001.460019.000.R.C.2011.000.10300). This database contains *ca.* 3000 mass values corresponding to these 6 main milk proteins, including genetic variants, splicing isoforms, post-translational modifications (phosphorylation, glycosylation) and the main degradation products (due to the action of plasmin, *i.e.* essentially γ-caseins and related proteose-peptones).

In order to establish reliable equations, only samples with a proteolysis rate lower than 20% were retained, *i.e.* 193 samples in cow milks, 152 samples in ewe milks, and 147 samples for goat milks. For each protein, outliers were removed by Grubb's test as indicated in the norm ISO 5725-2.

Calibration equations

For cow milk, the samples were divided into calibration and validation sets (n calibration=135 and n validation=58). For ewe and goat milk, a cross-validation was used, the sample number being a little low. The equations were developed by univariate PLS regression (Tenenhaus, 2002), data being centered but not reduced according to Bertrand et al. (2006). For each equation, optimal number of latent variables was chosen according to root mean square error of cross-validation (RMSEPcv). To improve equations and quality of estimation, a selection of wavelengths by genetic algorithm was performed before PLS regression in cow and ewe milk (Ferrand, 2010). The genetic algorithm (GA) used is the algorithm developed by Leardi (1998) which is specific to wavelength selection. Mutation rate, initial population, and number of variables selected in the solution of initial population were fixed to 1%, 30 and 5 respectively. GAs were performed with MATLAB 7.8 and PLS regressions were performed with the package PLS in R 2.8.1.

To compare and assess the equations, several statistical parameters were computed: mean, standard deviation (Sd), standard error of validation ($SE_{validation}$), validation coefficient of determination ($R^2_{validation}$) and the relative error ($SE_{validation}$/Mean (%)).

$SE_{validation}$ is defined as $\sqrt{\dfrac{\left(\sum\limits_{i=1}^{N}(\hat{y}_i - y_i)^2\right)}{N-k-1}}$

with N being the number of samples and k the number of latent variables introduced in PLS regression.

We considered that estimation was accurate enough and robust to be applied in routine, when the relative error was under 8%. For relative error in the range of 8 to 12%, we advised using these equations with caution. We chose to use this parameter rather than the $R^2_{validation}$ because this latter depends on the standard deviation of our population.

Results

The equation performances for cow milk and ewe milk are presented in table 1. The best results are obtained for ewe milk, where the relative error is lower than 5% for total casein and β-casein, and inferior to 10% for the other three individual caseins (κ, α_{s1}, α_{s2}). For whey proteins, we can estimate the total, but not in the detail, equation for α-lactalbumin presenting a very low R^2.

In cow milk, we have higher relative errors and lower R^2. Total casein, β-casein, α_{s1}-casein are correctly estimated. These results are comparable to Rutten *et al.* (2011), except for β-lactoglobulin for which a higher error was obtained. Differences could be due to the reference method used that was different between the two studies (LC-MS versus capillary zone electrophoresis). Depending on the method used, quantitative values could be more or less accurate.

By contrast, the accuracy is lower with goat milk. It may be due to the lower amount of total protein in goat milk and to its polymorphism at the α_{s1}-casein locus which is responsible for large quantitative variations in milk protein content as it is for fat content and its fatty acid composition (Mahé *et al.*, 1994).

Table 1. Fitting statistics of prediction models (g/100ml), independent validation dataset for cow milk and cross-validation for ewe milk (PLS regression only or genetic algorithm (GA) + PLS regression).

	Cow milk					Ewe milk				
	N^i	Mean	Std	relative error (%)	R^2	N	Mean	Std	relative error (%)	R^2
TP	58	3.341	0.307	0.98	0.99	147	5.080	0.772	0.38	1.00
Caseins	57	2.458	0.271	3.93	0.88	149	4.089	0.666	2.73	0.97
κ-CN	57	0.317	0.054	11.61	0.54	145	0.441	0.069	6.57	0.82
α_{S2}-CN	58	0.237	0.041	10.29	0.65	147	0.557	0.102	7.46	0.83
α_{S1}-CN	57	0.860	0.100	6.32	0.71	143	1.216	0.210	5.06	0.91
β-CN	57	1.037	0.130	5.91	0.78	147	1.834	0.315	4.14	0.94
Whey proteins	57	0.385	0.058	9.96	0.58	145	0.564	0.097	8.89	0.73
α-LA	57	0.123	0.018	10.91	0.48	143	0.152	0.029	16.45	0.26
β-LG	58	0.263	0.054	15.29	0.45	145	0.410	0.090	10.47	0.77

To validate these first equations, we have applied the ewe equations on the 127 040 ewe milk spectra of the PhenoFinLait database. Distributions are normal and means are coherent with the bibliography (table 2). These results confirm the equation abilities to estimate the protein contents on average, even if some verifications are still necessary.

Table 2. Estimation of proteins composition of ewe milks' from the PhenofinLait database, Descriptive statistics (PLS regression only or genetic algorithm (GA) + PLS regression).

	Mean	Std
TP	5,456	0,768
Caseins	4,399	0,68
κ-CN	0,465	0,07
α_{S2}-CN	0,592	0,131
α_{S1}-CN	1,375	0,217
β-CN	1,908	0,307
Whey proteins	0,64	0,089
α-LA	0,158	0,017
β-LG	0,472	0,085

Determination of protein composition in milk by mid-infrared spectrometry

Conclusions

These first results show it makes it possible to obtain accurate estimations for each casein in individual milk samples of ewe and cow.
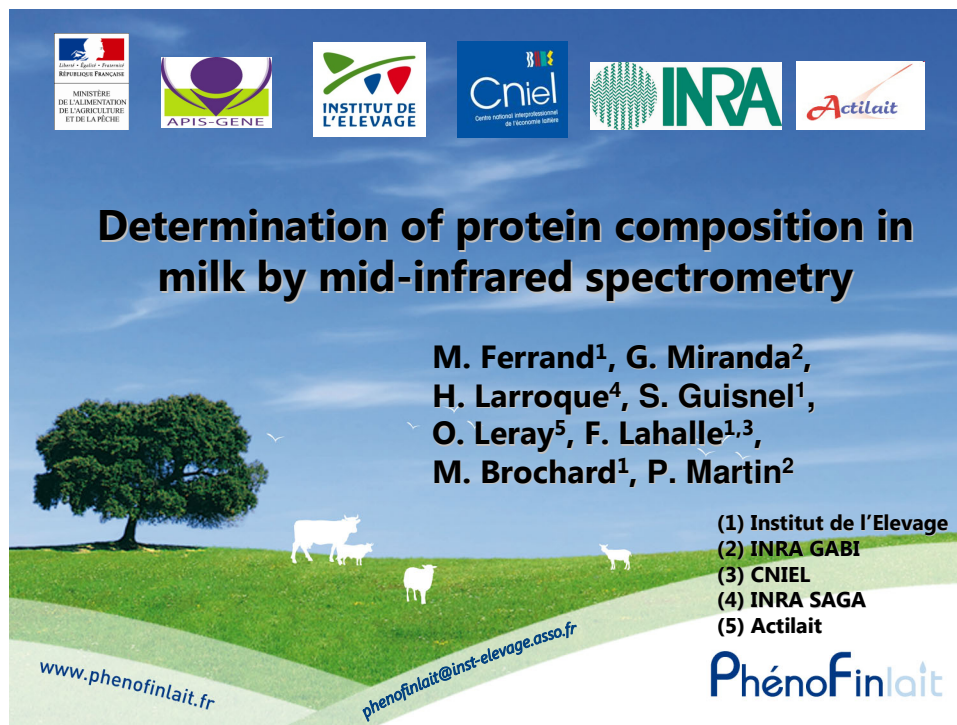
In goat milk, equations are clearly insufficiently accurate, probably due to the strong polymorphism existing at the α_{s1}-casein locus in that species, which may impact the accuracy of the results obtained with the reference method. Some works are still necessary to improve predictive equations for goats.

Further researches will focus on the improvement of the reference method, while increasing simultaneously the initial sampling size to get more accurate estimation of milk protein profile.

Progresses in the PhenoFinLait program are available on http://www.phenofinlait.fr/.

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Context

Milk = complex product with a lot of
components
        ↳ nutritional interests
        ↳ technological properties

→ no cheap and large scale easy to use
method to measure all milk components

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PhénoFinlait: aims

- Develop and control methods to analyze
fine milk composition easily
- Use the analytical development to
        - study genetic and feeding
management impact on milk composition
        - build up new tools to manage milk
composition (Dairy Herd Improvement (DHI)
and genomic)

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Major milk proteins

6 main milk proteins
α_{s1}-*Casein*
α_{s2}-*Casein*
β-*Casein*
κ-*Casein*
$\Bigg\}$ Caseins (*ca.* 80%)

β-*Lactoglobulin* (β-LG)
α-*Lactalbumin* (α-LA)
$\Big\}$ whey proteins (*ca.* 20%)

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Reference method (Miranda *et al.*)

Need to establish a reference method to identify and quantify major milk proteins:

➔ Liquid Chromatography + Mass Spectrometry (LC-MS)

Creation of a database of masses including genetic variants, splicing variants, post-translational modifications and main proteolysis products.

Bovine: 3000 referenced masses
Ovine: 1700 referenced masses

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LC-MS milk proteins profiling

Ovine milk

RP-HPLC

UV 214nm
EIC 600-3000 Da

Multicharged ions spectrum

Deconvolution

24546.0377

Splicing variant
α_{s2}-var A (del 34-42)
Exon 6 - skipping

25620.181
8 α_{s2}-var A

Bovine milk: 25 molecules identified

5 κ-Cn isoforms (1 to 3 glycosylation motifs)
2 κ-Cn isoforms (phosphorylation levels)
5 α_{s2}-Cn isoforms (phosphorylation levels)
2 α_{s1} and β-Cn isoforms (phosphorylation)
1 α_{s1}-Cn splicing variant (Del Q78)
2 β-Lactoglobulin genetic variants
1 α-Lactalbumin genetic variant
5 β-Cn fragments (γ-Cn and complementary fragments arising from plasmin proteolysis)

Method in routine

MIR spectra routinely obtained by milk recording laboratories for fat and protein percentage measurements

Spectrum from 75 cow milk samples (UE INRA Mirecourt + Domaine du Pin)
MilkoScan FT6000 (Foss Electric, Hillerod, Denmark)
LILANO (Milk recording laboratory)
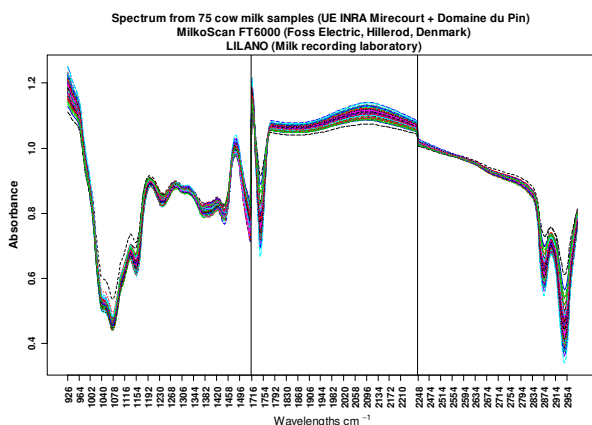
Already used to estimate FA and protein composition in cow milk (Soyeurt, 2006 – Rutten, 2011 – Bonfatti, 2011)

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Development of equations

- Traditionally by **PLS regression**
- **Pretreatments** can be useful to eliminate spectral variations → derivation to eliminate uncontrolled spectral variations (Soyeurt, 2011)
- Several authors have suggested **to apply a selection of variables before PLS regression** to improve results (Leardi 1998, Hoskuldsson 2001)
- Genetic algorithms already successfully used on IR data (Leardi 1998, Gomez-Carracedo 2007)
  → Previous study on fatty acids with good results (Ferrand, 2009)
- In genomic selection penalization method like LASSO, Ridge Regression or Elastic Net are used (Croiseau, 2011)

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Determination of protein composition in milk by mid-infrared spectrometry

Genetic algorithms method

- Optimization method based on evolutionary biology
- **Principle**: evolution of a population of solutions (=wavelength selection) using genetic operators like reproduction, mutation and selection
- **Objective**: obtain a population with the best solutions (=wavelength selection)

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Penalization method

Aim: to reduce the variance of estimators to guarantee the stability of the estimations

- Ridge Regression (RR): all the predictors are kept
- LASSO: some coefficients are set to zero and in presence of collinearity, only one predictor of the group is retained
- Elastic Net (EN): combination of RR and LASSO (two penalization parameters) → more flexible

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Samples analyzed

- **193 cow milk samples** from Holstein, Normande and Montbéliarde cows analyzed by MIR spectrometry and the reference method
- **153 ewe's milk samples** from Lacaune and Manech tête rousse
- **153 goat milk samples** from Saanen and Alpine

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Cow milk: selected wavelengths

	GA	LASSO	EN
Number of retained wavelengths	**8 to 83**	**4 to 29**	**22 to 68**

- 2272-1944 cm^{-1} band rarely selected
- 2970-2278 cm^{-1} and 2272-1944 cm^{-1} selected for most proteins

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Previous study				Sy,x /Mean (%)						
Cow milk (independant validation)	**N**	**Mean**	**Sd**	**PLS1**	**dérivée + PLS1**	**AG 1 tour + PLS1**	**AG 2 tours + PLS1**	**EN (α=0,5)**	**EN (α=0,5) + PLS1**	**LASSO + PLS1**
Caseins	58	2.457	0.269	3.93	3.72	3.88	3.85			
glycosylated κ-CN	57	0.11	0.032	26.4	24.12	26.87	25.99	28.47	28.49	26.97
κ-CN	57	0.316	0.052	11.61	10.89	13.42	13.15	14.05	14.56	14.59
α$_{S2}$-CN	58	0.237	0.041	11.25	10.43	10.29	10.59	11.43	11.76	11.64
α$_{S1}$-CN	58	0.861	0.099	6.32	6.86	5.47	6.31	6.37	6.97	6.65
β-CN	58	1.041	0.132	7.22	6.04	5.91	6.7	7.09	6.38	6.99
Whey proteins	58	0.387	0.06	9.96	9.64	13.67	9.35			
α-LA	57	0.123	0.018	11.8	10.90	10.90	10.91	12.92	13.6	13.5
β-LG	58	0.263	0.054	15.79	15.86	15.29	15.39	16.63	16.28	15.89

Ewe's milk (cross-validation)	N	Mean	Sd	Sy,x /Mean (%)			R^2_{CV}		
				PLS1	AG 1 tour + PLS1	AG tour + PLS1	PLS1	AG 1 tour + PLS1	AG tour + PLS1
Caseins	149	4,09	0,67	2,92	2,73	3,02	0,97	0,97	0,97
κ-CN	146	0,44	0,07	7,02	6,71	6,57	0,80	0,82	0,82
α_{S2}-CN	147	0,56	0,10	7,46	9,41	9,50	0,83	0,73	0,72
α_{S1}-CN	143	1,22	0,21	5,06	5,45	5,62	0,91	0,91	0,90
β-CN	148	1,84	0,32	4,67	4,14	4,33	0,93	0,94	0,94
Whey proteins	146	0,56	0,10	9,55	8,89	9,29	0,69	0,73	0,71
α-LA	144	0,15	0,03	17,42	17,12	16,45	0,20	0,21	0,26
β-LG	145	0,41	0,09	10,47	10,49	17,31	0,77	0,77	0,38

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Application of ovine equations on PFL MIR-database

g/100 ml	Mean	Std
TP	5,456	0,768
Caseins	4,399	0,68
κ-CN	0,465	0,07
α_{S2}-CN	0,592	0,131
α_{S1}-CN	1,375	0,217
β-CN	1,908	0,307
Whey proteins	0,640	0,089
α-LA	0,158	0,017
β-LG	0,472	0,085

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Conclusions

- In first place, to have robust equations, it seems fundamental to have a robust sample dataset with variability and accurate measurements by the reference method
- Gain of accuracy by reallocating the proteolysis
- To implement these equations at a large scale, it is also central to establish an harmonization system between laboratories (Leray *et al.,* 2011)

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Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

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Abstract

Milk coagulation properties (MCP) are fundamental in cheese production, particularly in countries where a large amount of milk is destined to cheese industry. Several studies have demonstrated the role of MCP on cheese yield and quality. Lasting years, a general worsening of MCP at the herd and animal level has been detected. The coagulation of milk is influenced by several factors such as type and quantity of clotting enzyme, acidity and calcium content of milk, and protein content and composition.

Milk coagulation properties are currently determined using several instruments, the most common being Reomether, Coagulometer, Formagraph, and Optigraph, which measure rennet coagulation time (RCT, min) and curd firmness after rennet addition (a_{30}, mm). Nevertheless these instruments have strong limitations for the use at population level, mainly because they are time-consuming, expensive and require skilled personnel. The Fourier Transform Mid-Infrared Spectroscopy (FTMIR) allows for a reduction of costs needed for the analysis, high throughput, and possibility of large-scale application, i.e., the implementation in milk recording programs.

In 2008 the feasibility to predict MCP using FTMIR was investigated in dairy herds located in north-east Italy and the results were encouraging; correlation coefficients for the prediction models of technological properties were comparable to those used for other novel traits (e.g., protein fractions and fatty acids). The northeast of Italy is characterized by a strong synergy among the dairy chain stakeholders (farms, dairy cooperatives, milk quality labs, animal breeding companies and research institutions).

Since 2009 several regional projects have been financed and coordinated by the University of Padova (Italy) with many stakeholders of the Veneto region dairy chain, achieving a consistent improvement in efficiency of the dairy sector. The projects aimed at studying the technological characteristics of milk through an innovative approach (from cow's milk to cheese), and it was developed through the implementation of MCP calibration models to a MilkoScan which routinely analysed individual and bulk milk samples from all the associated farms and dairies of the region.

Data of individual milk samples (about 200,000 records), mainly from Holstein-Friesian cows, and herd bulk milk samples (about 15,000 records) from the 3 major dairy cooperatives, were recorded. Genetic analysis was carried out on MCP and estimated breeding values were obtained. Bulk milk samples were used [1] to study the sources of variation of MCP at herd level focusing more on management and feeding characteristics, [2] to optimize the cheese production at dairy level according to technological aptitude of milk to be converted into cheese, and [3] to define new quality payment systems that take into account the MCP. Currently, the projects are undergoing and the opportunity to extend the regional prototype at national level and at different dairy species is under evaluation.

Keywords: mid-infrared spectroscopy, milk coagulation properties

Introduction

The dairy industry is more and more interested in improvement of MCP, as they affect the efficiency of cheese-making process (Aleandri *et al.*, 1989; De Marchi *et al.*, 2008; Wedholm *et al.*, 2006), and cheese yield and quality (O'Callaghan *et al.*, 2000). Milk coagulation properties are the result of several interacting factors such as chemical composition (fat, protein, and casein contents) and acidity of milk, somatic cell count, and calcium and phosphorus concentrations. Besides these characteristics, genetic aspects play an important role in determining MCP. Differences among breeds (Auldist *et al.*, 2004; De Marchi *et al.*, 2007) and among individuals within breed (Cassandro *et al.*,

2008; Ikonen *et al.*, 2004; Vallas *et al.*, 2010) have been reported in literature, suggesting that technological properties are heritable traits and can be genetically improved.

Traditionally, MCP have been determined by using time-consuming instruments able to process few samples per hour. To drastically reduce the time and costs of analysis of the reference methods, and to extend the MCP determination at population level, the Fourier Transform Mid-Infrared Spectroscopy (FTMIR) has been proposed as a fast, non-destructive and cheap method to assess MCP. De Marchi *et al.* (2009) indicated that only rennet coagulation time (RCT, min) can be satisfactorily predicted by FTMIR and Cecchinato *et al.* (2009) reported that FTMIR predictions can be proposed as indicator traits for the genetic enhancement of technological quality of milk.

Currently, the dairy industry is much interested (i) in understanding how different breeds perform under the same management conditions (e.g., within the same herd) and (ii) in the feasibility of using FTMIR for milk payment purposes. While studies have demonstrated that FTMIR can be used to improve MCP via selection, there is a lack of support that it can be used in milk payment schemes as the accuracy of prediction models is not large enough for this purpose (De Marchi *et al.*, 2009). Moreover, the prediction models have been developed using only samples that coagulate within 30 minutes from rennet addition.

Prediction of MCP by FTMIR

Coagulation properties of 356 samples of bovine milk were determined by the Formagraph (Foss Electric, Hillerød, Denmark) for 60 minutes in the laboratory of the Breeders Association of Veneto region (Padova, Italy). The testing-time of the analysis was set up at 60 minutes to investigate if milk not forming a curd within the conventional threshold of 30 minutes (Ikonen *et al.*, 1999; Cassandro *et al.*, 2008; De Marchi *et al.*, 2009; Tyrisevä *et al.*, 2003) showed coagulation aptitude after this time. Measured traits were RCT (the interval, in minutes, from the addition of the clotting enzyme to the beginning of coagulation), curd-firming time (k_{20}, the interval, in minutes, from the beginning of coagulation to the moment the width of the graph attains 20 mm), and curd firmness 30 minutes (a_{30}) and 60 minutes (a_{60}) after rennet addition. Besides measures of MCP, FTMIR spectra were collected on milk samples using a Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark) within 4 hours from reference analysis.

Prediction models of MCP were performed using partial least square regression analysis confirmed using cross-validation method. The effectiveness of validation models was assessed using the standard error of cross-validation (SE_{CV}) and the coefficient of determination of cross-validation (1-VR). The ratio performance deviation (RPD) and the range error ratio (RER) were calculated to provide indications on the practical utility of prediction models.

The most accurate models were those for RCT, k_{20}, and a_{30} with 1-VR of 0.76, 0.72, and 0.70, respectively (Table 1). Less favorable results were obtained for a_{60}. The prediction models allowed the determination of samples with RCT longer than 30 minutes (Figure 1), which were not taken into account in a previous study by De Marchi *et al.* (2009). The practical utility indexes combined with 1-VR and SE_{CV} suggest that models for MCP can be adopted by the dairy industry for payment of milk as well as for genetic purposes.

Table 1. Fitting statistics of prediction models for milk coagulation properties (RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 minutes after rennet addition; a_{60} = curd firmness 60 minutes after rennet addition).

Trait	#L[1]	SE_{CV}[2]	1-VR[3]	RPD[4]	RER[5]
RCT, min	15	7.05	0.76	2.03	25.22
k_{20}, min	12	3.54	0.72	1.86	14.22
a_{30}, mm	17	7.68	0.70	1.80	28.20
a_{60}, mm	12	7.26	0.42	1.26	31.80

[1]#L = number of modified partial least square factors used in the calibration. [2]SE_{CV} = standard error of cross-validation. [3]1-VR = coefficient of determination of cross-validation. [4]RPD = SD/SE_{CV}. [5]RER = SE_{CV}/range.
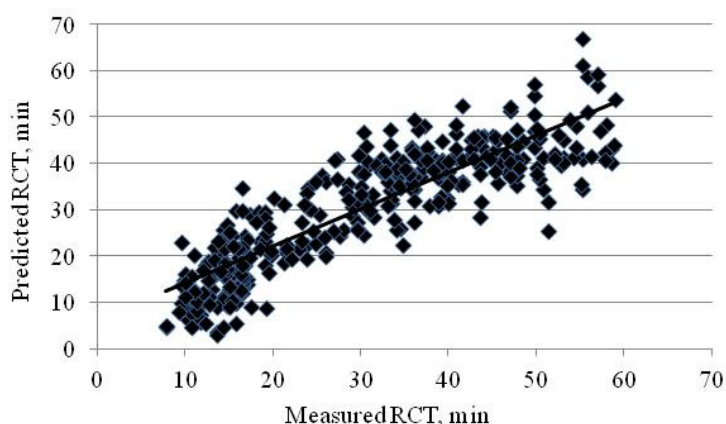
Figure 1. Scatter plots of predicted (y-axis) vs. measured (x-axis) rennet coagulation time (RCT).

Coagulation properties of bulk milk samples

A total of 1,508 bulk milk samples were collected between June 2008 and November 2009 from 436 dairy cow herds which delivered milk to 4 dairy cooperatives. Milk quality traits (casein content, fat content, titratable acidity, somatic cell count, and bacterial count) and MCP were assessed in the laboratory of Veneto region (Thiene, Italy). An analysis of variance was performed on MCP traits using the GLM procedure of SAS (SAS, 2008). The linear model included the fixed effects of dairy cooperative, herd nested within dairy cooperative, and year and season of sampling. Besides these factors, class effects of casein content, fat content, titratable acidity, somatic cell count and bacterial count were also tested.

Means (SD) of RCT, k_{20}, and a_{30} were 18.83 (3.68) min, 6.85 (1.92) min, and 26.97 (8.12) mm, respectively. Bulk milk non-coagulating within 30 minutes represented 4% of total samples; this value is lower than that (9.7%) from Cassandro et al. (2008) on individual Holstein-Friesian milk samples.

Table 2. Results from analysis of variance for milk coagulation properties measured on herd milk samples (n = 1,508).

Effect	df	RCT, min		k_{20}, min		a_{30}, mm	
		F	P-value	F	P-value	F	P-value
Dairy cooperative[2]	3	25.03	<0.001	9.36	<0.001	25.57	<0.001
Herd (within dairy cooperative)	416	1.86	<0.001	1.57	<0.001	1.83	<0.001
Year of sampling	1	19.08	<0.001	1.12	0.290	0.07	0.797
Season of sampling	3	13.75	<0.001	2.66	0.047	1.51	0.211
Casein, %	4	0.71	0.585	4.84	0.001	5.88	<0.001
Fat, %	4	0.58	0.676	1.06	0.376	1.47	0.209
Titratable acidity, °SH/50mL	4	14.31	<0.001	4.78	0.001	13.63	<0.001
Somatic cell count, cells/ml	4	2.31	0.056	1.13	0.339	0.97	0.422
Bacterial count, cells/ml	4	2.48	0.042	1.70	0.148	1.56	0.183
R^2		0.52		0.52		0.52	
RMSE[3]		3.03		1.65		6.72	

[1]RCT = rennet coagulation time; k_{20} = curd-firming time ; a_{30} = curd firmness 30 minutes after rennet addition.
[2]Tested on herd (within dairy cooperative) variance.
[3]RMSE = root mean square error.

Milk coagulation properties were strongly influenced by dairy cooperative and herd (Table 2), suggesting the existence of different feeding and management conditions. As expected, the chemical composition and acidity of milk had a large influence on MCP (Table 2). In particular, MCP improved with increasing values of casein and titratable acidity. The season of sampling had an impact on RCT and k_{20} (Table 2) and better results were obtained during summer, as previously reported by Chlàdek et al. (2011).

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

MCP in multibreed herds

Thirty-nine multibreed herds from Veneto region enrolled in monthly test-day milk recording were selected to evaluate the performance of Holstein-Friesian (HF), Brown Swiss (BS), and Simmental (SI) breeds under similar environmental conditions. Average breed contribution within each herd was calculated. The 39 selected herds reared at least two of the aforementioned breeds.

A total of 8,525 individual milk samples collected between September 2011 and February 2012 were analyzed for fat and protein contents, somatic cell count, RCT and a_{30} in the laboratory of the Breeders Association of Veneto region (Padova, Italy) using Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark). Besides quality traits, daily milk yield was also available. Casein index was calculated as the ratio between casein content and protein content, and somatic cell score was obtained via log-transformation of somatic cell count. Samples that did not coagulate within 30 minutes were discarded from the dataset, as well as samples exceeding 4 standard deviations from the mean of each trait.

Data were analyzed through a generalized linear model using the MIXED procedure of SAS (SAS, 2008). The model included the fixed effects of month of test-day (6 levels), parity (4 classes, the last being an open class), days in milk (12 monthly classes, the last being an open class), herd, breed, interaction between parity and breed, interaction between days in milk and breed, and the random effects of cow (within breed) and residual.

Table 3. Least squares means of milk quality traits and MCP across breeds.

Trait	Breed		
	Holstein-Friesian	Brown Swiss	Simmental
Milk yield, kg	27.5	24.1	23.6
Fat, %	3.83	4.25	4.05
Protein, %	3.47	3.76	3.64
Casein, %	2.74	2.99	2.88
Casein index	78.8	79.9	79.1
SCS, score	3.44	3.16	2.96
RCT, min	21.0	19.1	20.2
a_{30}, mm	20.8	26.8	23.6

Holstein-Friesian produced more milk per day than BS and SI cows (Table 3). For fat, protein, and casein contents, and casein index, the best results were obtained for BS, followed by SI and HF. Somatic cell score was lower for SI than BS and HF cows. Regarding MCP, BS produced milk with the shortest RCT and the highest a_{30}, whereas HF showed the worst technological properties (Table 3). Findings are consistent with previous reports of De Marchi *et al.* (2007), where breeds were compared on bulk milk samples from single-breed herds. In the present study, individual samples from multibreed herds were collected, so that the effect of different breeds on MCP could be estimated, under similar rearing conditions. Results confirmed that HF suffers for scarce MCP, whereas milk from BS cows has good aptitude to coagulate. Finally, MCP of SI cows were intermediate between BS and HF.

Conclusion

In Veneto region (northeast Italy), the dairy industry is making notable efforts to improve the technological properties of bovine milk. The link among the dairy chain actors, namely farmers, dairy cooperatives, milk quality labs, artificial insemination companies, and research institutions, facilitates the research on MCP at the different stages of the chain.

The FTMIR demonstrated its potential to predict RCT, k_{20}, and a_{30} of individual samples with enough accuracy to allow the use of this technique for routine recording of MCP, for selection goals and for milk payment purposes. This is an important result, as a large amount of milk in Italy is used to produce high-quality cheeses.

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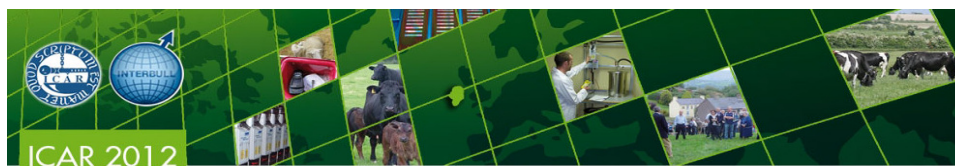
Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

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DAFNAE - University of Padova - Italy

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2. Milk Coagulation Properties (MCP)
   1. What is MCP
   2. How to measure MCP
   3. Researches on MCP
3. Prediction of MPC by FTMIR
4. Predicted values of MCP: genetic applications
   1. Breed effect on MCP
   2. Genetic Improvement of MCP
5. MCP and Dairy industry
   1. MCP and cheese yield
   2. MCP and milk payment system
6. Conclusions & Perspectives

Introduction

Cheese production is growing worldwide (International Dairy Federation, 2010) with a +2% of rate / year (SCHMIT and KAISER, 2006).

Italy 70% of available milk used for manufacturing typical cheeses, in particular PDO products (Pieri, 2011).
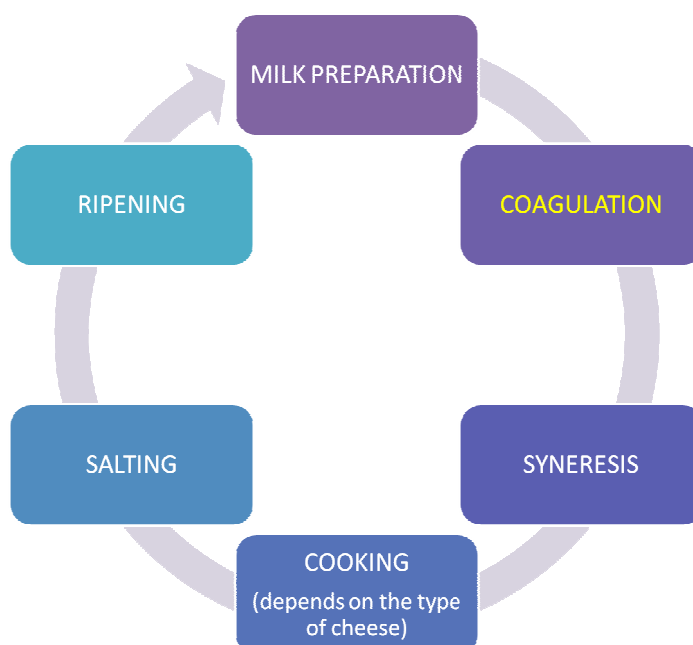
More than 83% of Italian cow milk production is localized in the North regions.
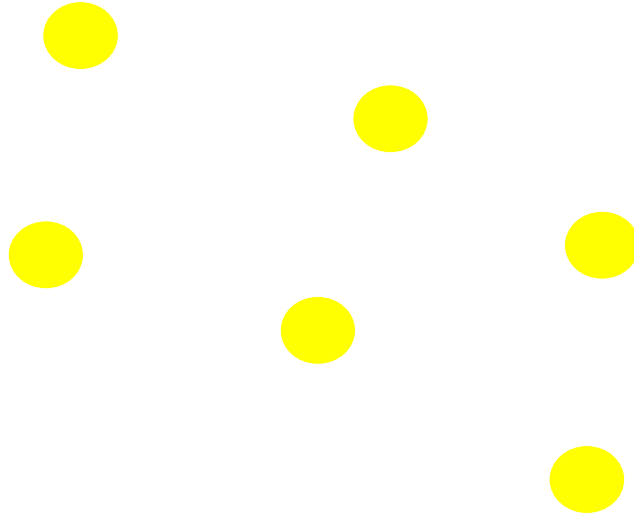
The Veneto Region is 3rd producer of cow milk in Italy.

Since 2008 a regional project "Distretto Lattiero Caseario Veneto" had linked dairy industry stakeholders.
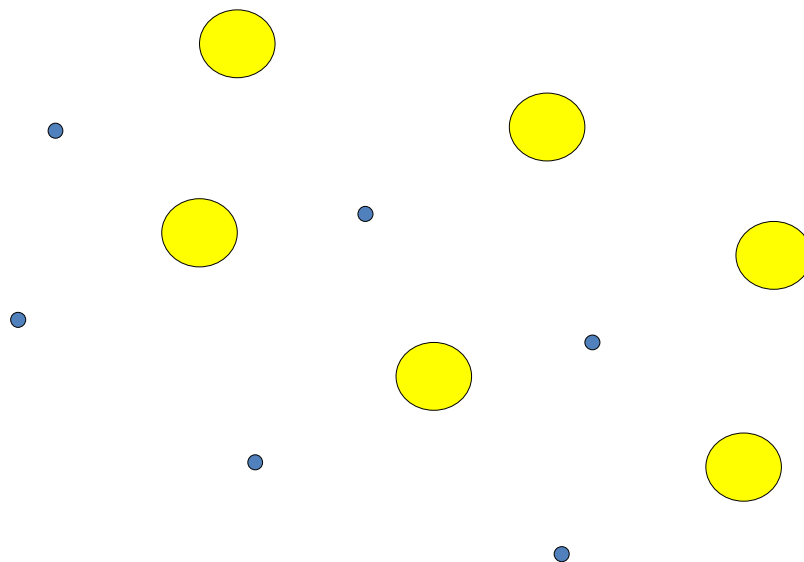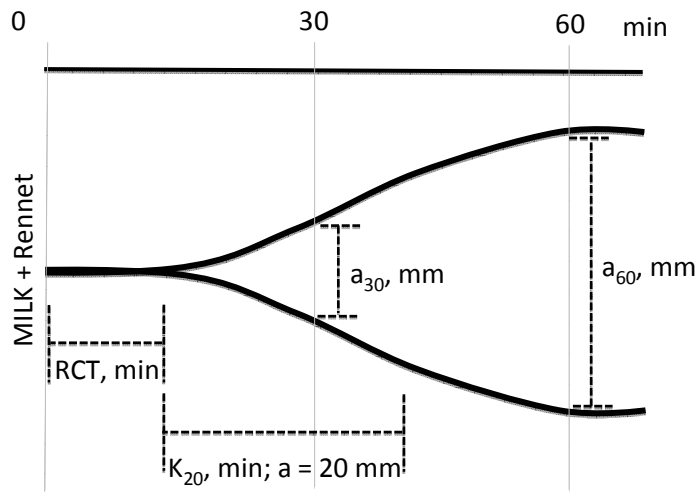
Cheese Making Steps

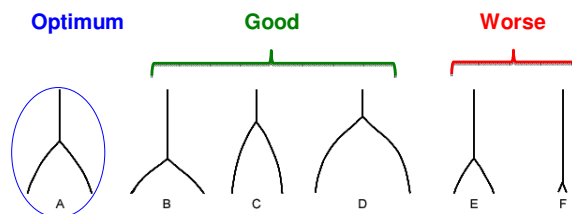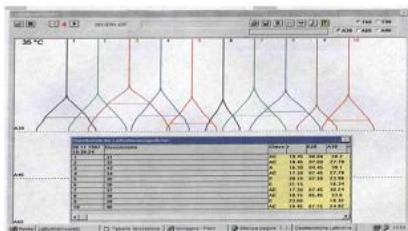## Stability of milk due to the crown of k-casein

Action of chimosine of rennet
cut K-casein making the coagulation

Milk Coagulation Properties: scheme

Milk Coagulation Properties: output

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

MCP: Why and How measured?

Cheese processing, cheese yield, cheese composition and quality → dairy efficiency.

[Aleandri et al., 1989; Ng-Kwai-Hang et al., 1989; Martin et al., 1997; Walsh et al., 1998; Ikonen et al., 1999; Summer et al., 2003; Wedholm et al., 2006; De Marchi et al., 2008].

Mechanic and optical methodology has been studied.

[Laporte et al., 1998; O'Callaghan et al., 2002; Klandar et al., 2007; Cassandro et al., 2008; Dal Zotto et al., 2008; Pretto et al., 2011].

The common method is the lactodynamograph.

Variation Sources of MCP

1. Type of instrument and methodology of analyses [Pretto et al., 2011].

2. Milk quality composition. e.g. protein composition, titratable acidity, somatic cell content [Okigbo et al. 1985; Politis and Ng-Kwai-Hang 1988; Formaggioni et al., 2001; De Marchi et al., 2007].

3. Species, breed, individuals [Macheboeuf et al., 1993; Auldist et al., 2002; Bencini, 2002; Park et al., 2007; De Marchi et al., 2007 and 2008; Martin et al., 2009].

4. Lactation stage

5. Herd and environmental conditions

6. Exploitable genetic variation exist for MCP [Ikonen et al., 1999; Tyriseva et al., 2004 and 2008; Cassandro et al., 2008; Comin et al., 2008; Vallas et al., 2010]

Prediction of MCP by FTMIR

Prediction of MCP by FTMIR

1. Fast / No destructive / Easy to use.

2. Largely used by milk labs to assess milk quality and for milk payment or for routine milk recording analyses.

3. Absorptions of IR at frequencies correlated to the vibrations of specific chemical bonds within a molecule (Coates, 2000).

C-H bending
640-1500 cm^{-1}

Fatty acid carbonyl group
1736; 1805;1745 cm^{-1}

DE MARCHI ET AL.

Protein
1570-1550 cm^{-1}

Lipids
3000-2800 cm^{-1}

C-O stretching
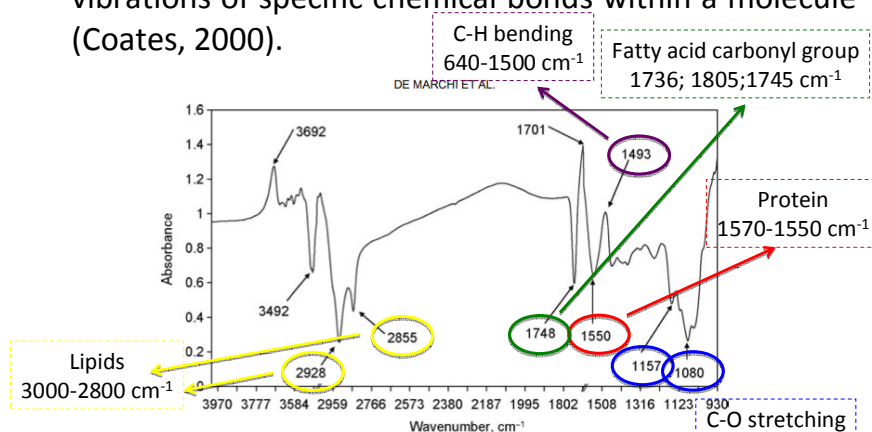
Milk/Cheese traits by FTMIR: applications

1. Fatty acids [Soyeurt et al., 2006; Rutten et al., 2009; De Marchi et al., 2011; Soyeurt et al., 2011].

2. Protein composition [Lynch et al., 2006; De Marchi et al., 2009; Rutten et al., 2011].

3. Acetone [Heuer et al., 2001].

4. **MCP** [Dal Zotto et al., 2008; De Marchi et al., 2009; De Marchi et al., 2012].

5. Body energy status [McParland et al., 2011].

6. Sensory texture traits of cheese [Fagan et al., 2007].

7. Melamin [Foss, personal communication].

Prediction of MCP by FTMIR

2009 →   2010÷2011 →   2012

- 1,200 individual milk samples.
- NO preservative / Analysis within 3 hours.
- Computerized renneting meter (Polo Trade).
- Spectra from Milko-Scan FT120.
- RCT (14.96±3.84 min) and a_{30} (41.7±8.76 mm).
- RCT = R^2_{cv} of 0.62; a_{30} R^2_{cv} of 0.37.

RCT model → allows the discrimination between high and low value.

a_{30} → no satisfactory prediction.

K_{20} → no prediction.

Low range of variability (no samples RCT > 29.5 min).

Reference methods / Lab conditions.

De Marchi et al., 2009 J. Dairy Sci. 92:423–432

Prediction of MCP by FTMIR

2009 →   **2010÷2011 →**   2012

- 850 milk samples (individual HF and <u>bulk</u>).
- <u>Preservative</u> / Analysis within <u>3 days</u>.
- <u>Formagraph</u> (Foss Electric).
- Spectra from <u>Milko-Scan FT6000</u>.
- RCT (19.82±4.59 min) and a_{30} (22.63±10.95 mm).
- RCT = R^2_{cv} of 0.66; a_{30} R^2_{cv} of 0.70.

RCT and a_{30} → quite satisfactory prediction models.

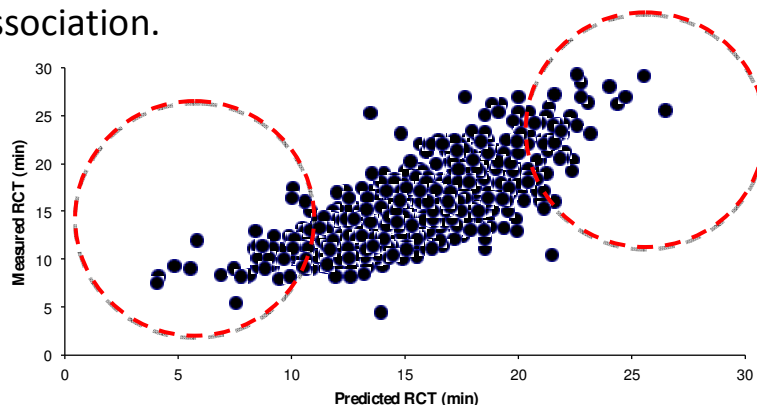K_{20} → no prediction.

no sample RCT > 29.5 min.

Limited range (RCT from 8 to 25 min).

Prediction of MCP by FTMIR

2009 →   **2010÷2011 →**   2012

Summer 2011 - RCT and a_{30} models were installed in Milko-scan FT6000 of Regional breeder association.

Difficult to predict RCT < 8 and > 24 minutes

Prediction of MCP by FTMIR

2009 → 　　　　2010÷2011 → 　　　　**2012**

- 350 milk samples (mainly HF).
- <u>Formagraph</u> (Foss Electric).
- RCT, K_{20}, a_{30} and a_{60}.
- Milko-Scan FT6000.
- RCT (range from 7.55 to 59.00 min)

	RCT, min	K_{20}, min	a_{30}, mm	a_{60}, mm
R^2_{cv}	0.76	0.72	0.70	0.42
RPD	2.03	1.96	1.80	1.26
RER	25.22	14.22	28.20	21.20

1-VR = coefficient of determination of cross-validation.
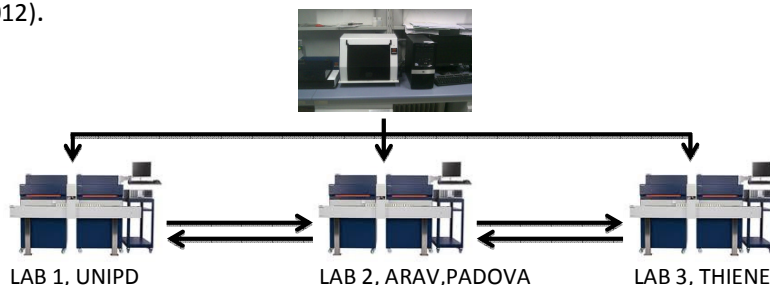RER = SEC_{CV}/range. RPD = SD/SEC_{CV}.

De Marchi et al., 2012

Prediction of MCP by FTMIR

2009 → 　　　　2010÷2011 → 　　　　**2012**

Ring test is carrying out by three labs, twice per month using individual and bulk milk samples to reduce the bias between FTMIR instruments and reference data and among FTMIR instruments.

The correlation between MCP values measured by FT6000 and MCP measured by Formagraph in routinely condition range from 0.88 to 0.91 for RCT and a_{30}, respectively (update to April 2012).

LAB 1, UNIPD　　　　LAB 2, ARAV,PADOVA　　　　LAB 3, THIENE

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability
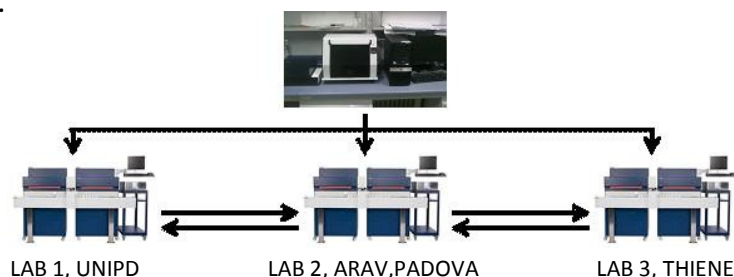
Prediction of MCP by FTMIR
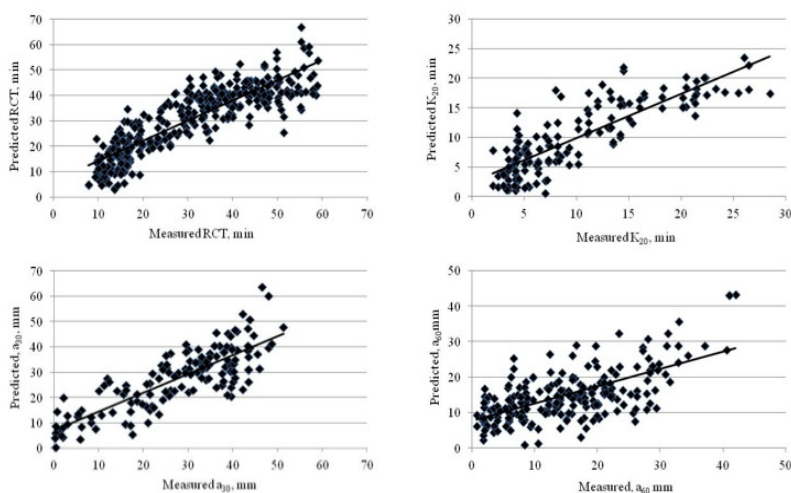
2009 → 2010÷2011 → **2012**

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The correlation between MCP values measured by FT6000 and MCP measured by Formagraph in routinely condition range from 0.88 to 0.91 for RCT and a_{30}, respectively (update to April 2012).

LAB 1, UNIPD   LAB 2, ARAV,PADOVA   LAB 3, THIENE

Prediction of MCP by FTMIR

Scatter plots of predicted (y-axis) on measured (x-axis) (RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 minutes; a_{60} = curd firmness at 60 minutes).

De Marchi et al., 2012

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

Predicted MCP Values: applications

Predicted MCP Values: genetic applications

In Veneto region about 25,000 controlled cows/month

RCT and a_{30} measured using Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark).

Effect of cow breed on MCP in multibreed herds

1.39 mixed herds were selected.

2.11,978 individual milk samples collected from 3,279 cows during monthly test-days between September 2011 and May 2012.

Predicted MCP Values: genetic applications
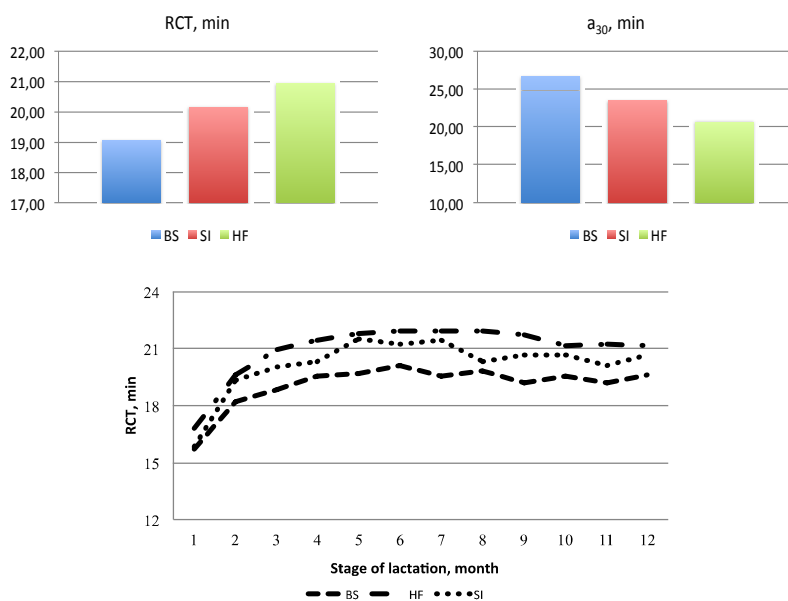
Predicted MCP Values: genetic applications

Genetic parameters for MCP in Holstein Friesian

312 herds - 3,488 HF cows - 140 bulls

	Mean	SD	Range	h^2	SE
Parity, n	1.9	1.1	1-10		
Days in milk, d	198	130	5-982		
Milk Yield, kg/d	30.90	9.60	3.8-88.0	0.12	0.04
Protein, %	3.40	0.40	2.29-5.87	0.20	0.02
Fat, %	3.72	0.75	1.50-8.75	0.29	0.06
SCS, punti	3.08	1.94	-1.64-10.72	0.03	0.02
pH	6.66	0.10	6.17-7.13	0.19	0.04
RCT, min	**19.50**	**5.00**	**2.90-30.00**	**0.17**	**0.05**
a_{30}, mm	**25.00**	**12.50**	**1.00-64.3**	**0.20**	**0.04**

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

Measured vs. Predicted MCP: Genetic analysis

1,200   Brown   Swiss   cows,   50   sires,   30   herds.
Measured MCP: RCT and a_{30} measured by Coagulometer
*Predicted MCP: RCT and a_{30} predicted by Milko-scan FT120*

	RCT, min		a_{30}, mm	
	Measured	Predicted	Measured	Predicted
R^2 MIRS	0.64		0.49	
Mean	15.1	14.9	41.5	41.7
σ^2_a	4.9	3.7	19.4	17.2
σ^2_h	1.7	1.5	9.4	5.3
σ^2_e	9.5	4.6	20.6	20.0
h^2	32	37	24	35
r_g	94		77	

De Marchi et al. 2009. J. Dairy Sci. 92:423-432
Cecchinato et al. 2009 J. Dairy Sci. 92:5304-5313

Predicted MCP Values: genetic applications

Genetic parameters for MCP in Holstein Friesian
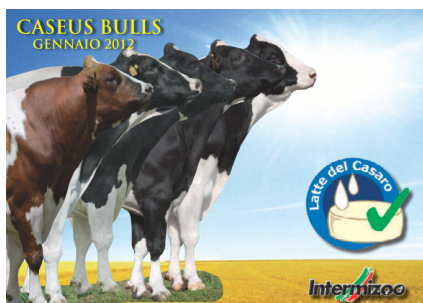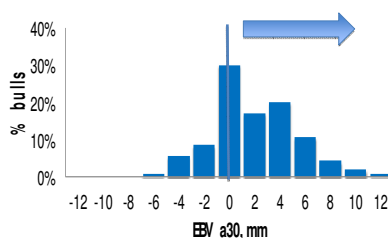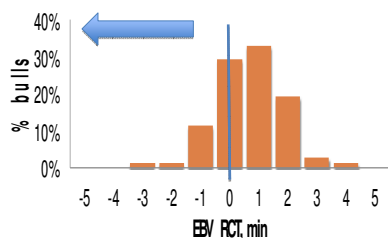
Top 12 HF BULLS for MCP

ACTIVE	PRINCE
BROSIO	PURPOSE
DUKO	QUASIMO
LAMBRO	SITTAX
MISIS	TABAIBA
PASSIRIO	WATHA

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

MCP and Dairy Industry: Variation Sources

1,508 bulk milk samples.

436 dairy herds.

4 dairy cooperatives (Lattebusche, Latteria di Soligo, Latterie Trevigiane, Latterie Vicentine).

2 years of sampling.

Table 1: Results from ANOVA for milk coagulation properties of bulk milk samples.

Effect	Trait[1]					
	RCT, min		k_{20}, min		a_{30}, mm	
	F	*P*-value	F	*P*-value	F	*P*-value
Dairy cooperative[2]	25.03	<0.001	9.36	<0.001	25.57	<0.001
Herd (within dairy cooperative)	1.86	<0.001	1.57	<0.001	1.83	<0.001
Year of sampling	19.08	<0.001	1.12	0.290	0.07	0.797
Season of sampling	13.75	<0.001	2.66	0.047	1.51	0.211
Casein, %	0.71	0.585	4.84	0.001	5.88	<0.001
Fat, %	0.58	0.676	1.06	0.376	1.47	0.209
Titratable acidity, °SH/50mL	14.31	<0.001	4.78	0.001	13.63	<0.001
Somatic cell count, cells/mL	2.31	0.056	1.13	0.339	0.97	0.422
Bacterial count, cells/mL	2.48	0.042	1.70	0.148	1.56	0.183
R^2	0.52		0.52		0.52	
RMSE[3]	3.03		1.65		6.72	

MCP and Dairy Industry: Variation Sources
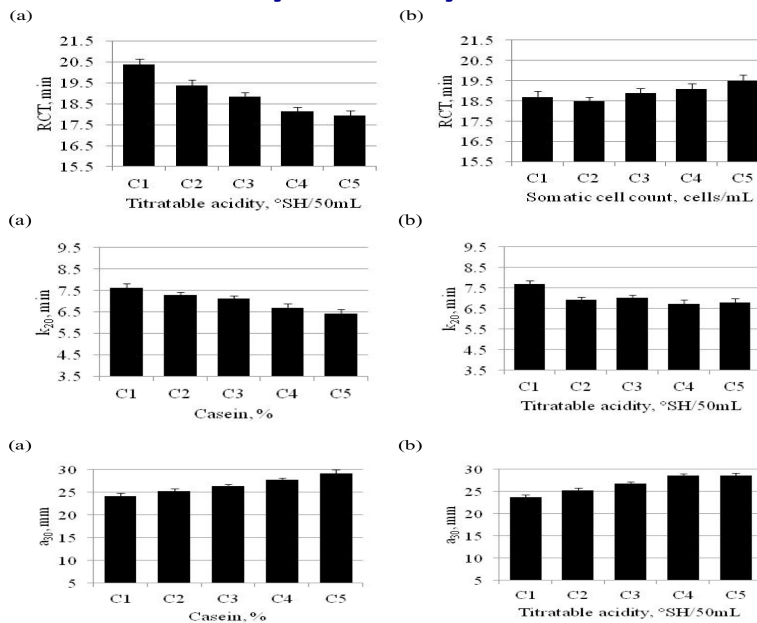
MCP and Dairy Industry: Cheese Yield

	Cheese yield		
Effect[1]	df	SS	P-value
Cheese-making day	11	1.246	<0.0001
Milk fat, %	2	0.525	<0.0001
Milk protein, %	2	0.446	0.0002
TA, SH°/50 mL	2	0.383	0.0006
a30, mm	2	0.175	0.0277

Curd firmness (mm)
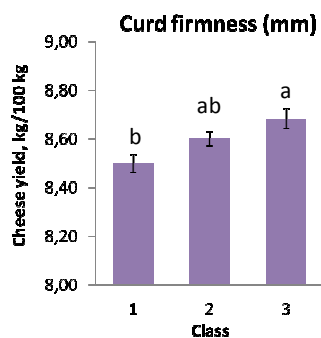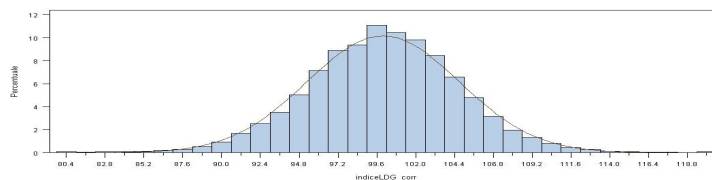

Mean per class: 20.52 - 26.05 - 31.88

Pretto et al. - Submitted

MCP and Milk Payment System

TOTAL INDEX that combine RCT and a_{30}

$$= 100 + \left[\left(\frac{A30 - \text{mean } a_{A30}}{sd \; A30} \right) * 2.5 - \left(\frac{R - \text{mean } r}{sd \; R} \right) * 2.5 \right]$$

Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

MCP and Economic Value of MCP under different milk payment systems

Relative Economic Value for the traits standardized
by genetic standard deviation (Pretto et al. 2010)
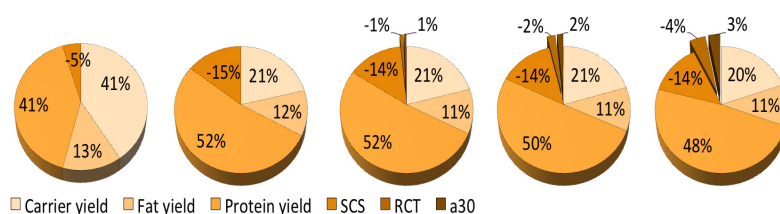
The weight for MCP ranged from 1.6 to 7.4 % (sum of absolute weight for
RCT and $_{a30}$), when the effect on cheese yield ranged from 1 to 5 % from the
worst and the best MCP.

Conclusion and Implication

Milk Coagulation Properties (MCP) are depleting and are an
important target for improvement in the dairy sector.
Several sources of variation of MCP were detected and quantified.

MIR models showed good predictions for MCP.

The MCP are fairly good for predicting in cheese yield (e.g. Grana
Padano).

The Economic Value of MCP should be about 7-8% of the milk price
and it is likely to increase if will increased consumption of dairy
products and if it will worsen the characteristics of dairy milk.

The development of MCP are indispensable for italian dairy chain.

The model and methods applied for these traits (MCP) and
research experience of the Veneto dairy chain might be applied for
more novel milk traits with the aim to design a new italian dairy
system.

Acknowledgement

Dott. Mauro Penasa
Dott. Francesco Tiezzi
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Dott.ssa Alba Sturaro
Dott.ssa Lucia Ancillotto

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Dott. Gianfranco Fait
Dott. Marco Cesari

THANK YOU
for
your ATTENTION

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk

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Abstract

This work is performed in the framework of the OPTIMIR project. It aims to develop innovative farm management web applications based on the use of infrared spectroscopy analysis of milk to enable a sustainable and profitable management of the milk production. Mid Infrared (MIR) spectroscopy has been the method of choice worldwide for quality control during routine milk testing. It allows a fast, non destructive and screening quantification of milk chemical properties, as the content of fat, protein and lactose.

In this project, the MIR spectrum will be used with a different purpose and will be considered as a reflexion of cows' state in order to obtain indicators concerning fertility, health and feeding among others. This innovative approach of using MIR needs the support of important spectral databases associated to reference values for each of the properties to be studied. For this reason, a large number of commercially available MIR spectrometers from different manufacturers installed in different laboratories from four different countries were used. Because of differences of the instrumental responses between different MIR spectrometers, spectra obtained on one instrument cannot readily be compared to a library acquired on a different instrument. Moreover, the use of calibration models developed on an instrument with MIR spectra obtained on another instrument will usually lead to an increased uncertainty of the prediction model. Then, spectral corrections adapted to each instrument (standardization) are needed.

In this work, the piecewise direct standardization (PDS) has been used in order to reduce the inherent instrument to instrument variability. The obtained results have shown a very good correlation between all the spectra and repeatable results across instruments, which is an indication that a common database could be constructed and would permit to develop MIR breeding tools which should be easily implemented in practice in many different countries.

MERGING OF SPECTRAL DATASETS FROM DIFFERENT MIR INSTRUMENTS USED IN THE ROUTINE ANALYSIS OF MILK
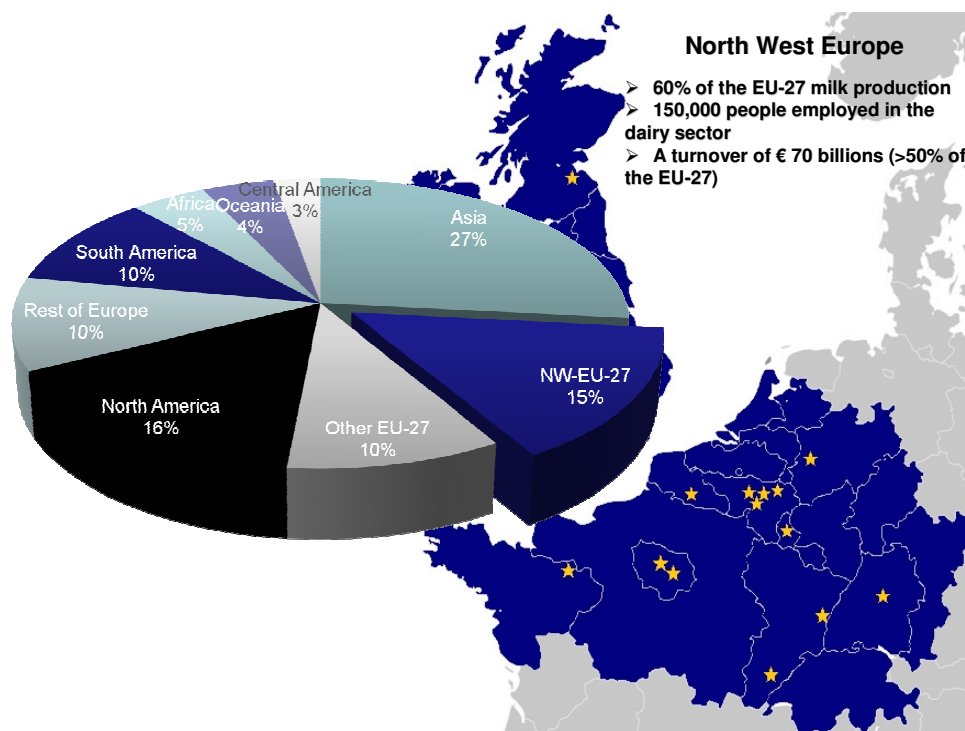
Juan Antonio Fernández Pierna, Clément Grelet
Frédéric Dehareng, Vincent Baeten & Pierre Dardenne
Walloon Agricultural Research Centre, CRA-W
Valorisation of Agricultural Products dpt
Gembloux, Belgium

Cork – 28 May 2012

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk

The OptiMIR Project

17 partners and 1 sub-contracting partner / 6 countries

Milk recording organizations	Country
AWE asbl	BE
Chambre régionale Agriculture Alsace	FR
ADECL62 (Pas-de-Calais)	FR
CLASEL (Sarthe & Mayenne)	FR
SCL25 (Doubs et territoire de Belfort)	FR
France Conseil Elevage	FR
LKV Baden-Württemberg	DE
LKV Nordrhein-Westfalen	DE
National Milk Recording	UK
Irish Cattle Breeding Federation	IR
CONVIS	LU

Research Units	Country
Institut de l'Elevage	FR
Gembloux Agro-Bio Tech (ULg)	BE
CRA-W (DVPA)	BE
TEAGASC	IR
Scottish Agricultural College	UK
University of Hohenheim	DE

Laboratory	Country
Comité du Lait asbl	BE

AIMS

To improve the profitability and sustainability of the dairy sector by providing milk producers with innovative standardized management tools based on association between MIR milk records and cows' status.

To reduce the costs of production through improved daily herd management.

To bring opportunities to access competitive markets by measuring quality traits linked to higher added value (e.g. low-cost measure of food label claims).

costs of feeding with energetic balance indicator

veterinary costs with early diagnosis of mastitis

costs of semen straws with insemination predictor

To decrease the impact on the environment (quantification of methane and nitrogen production).

Mid-Infrared (MIR) spectra of milk
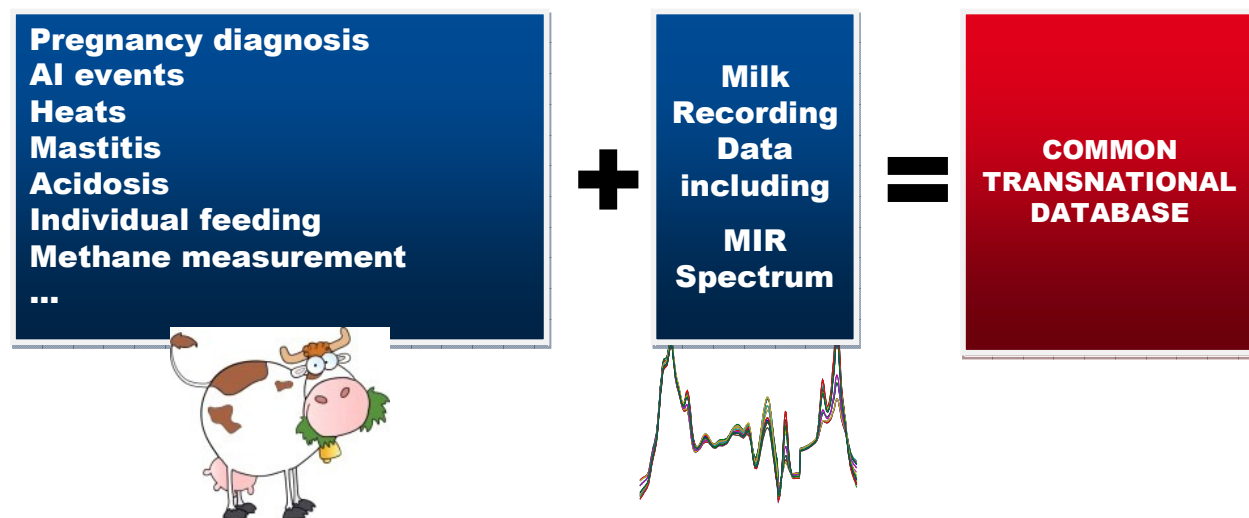# =
Mirror of cow's status

Milk MIR spectrum
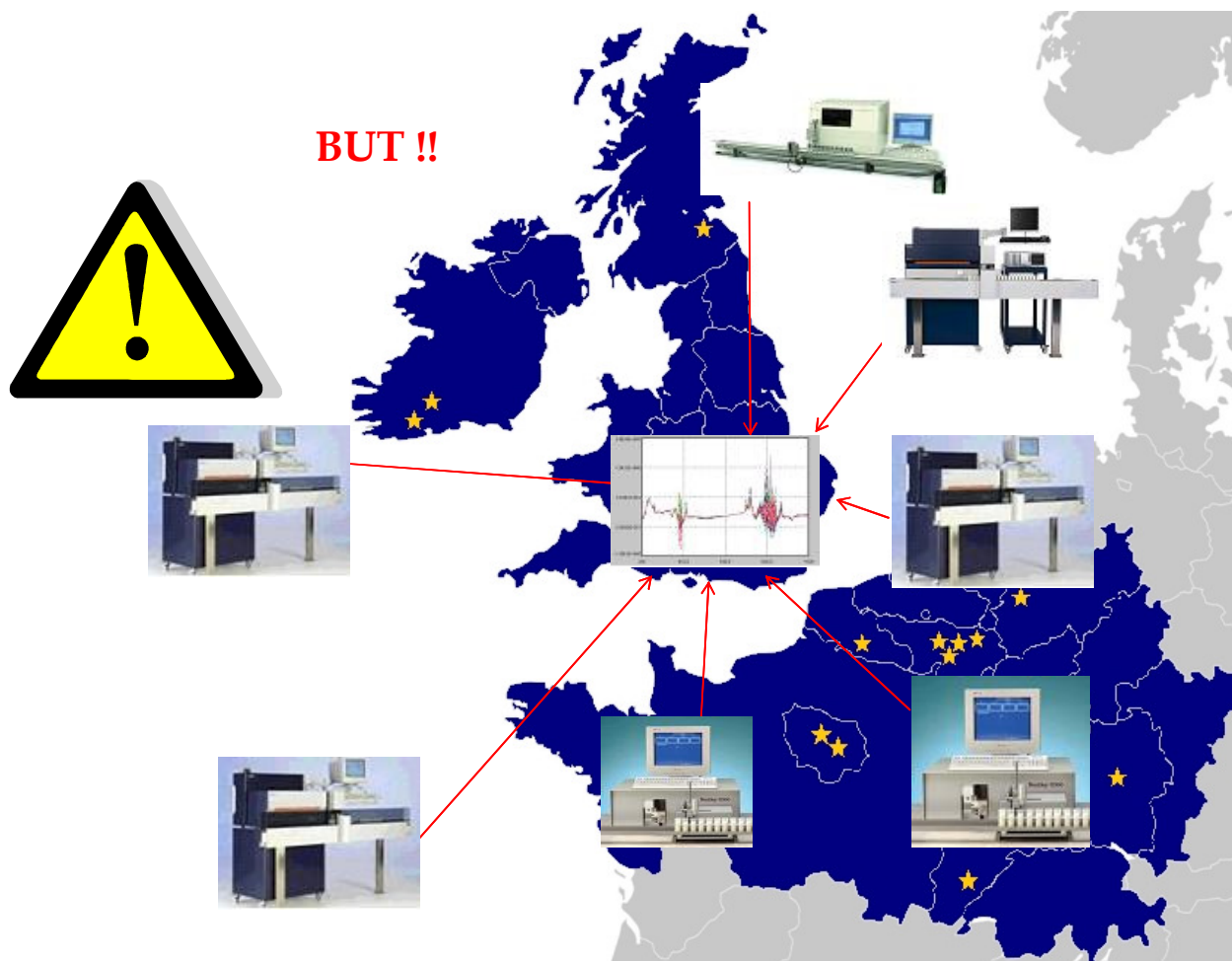**for each individual cow**
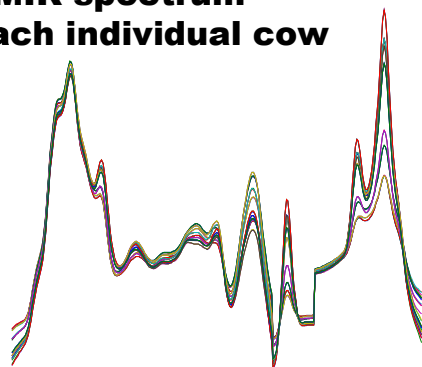
- **FERTILITY**
  Ex. : Pregnancy

- **FEEDING**
  Ex. : Energy Balance

- **ANIMAL HEALTH**
  Ex. : Mastitis

- **ENVIRONNEMENTAL IMPACT**
  Ex. : CH_4

To pool the resources of Milk Recording Organizations to have a **common transnational database** coupling:

- Physiological data of the cows
- with the related Milk Recording data
- and the standardized Spectrum Information (1060 values for each wavelength for a Foss instrument / record)

Pregnancy diagnosis
AI events
Heats
Mastitis
Acidosis
Individual feeding
Methane measurement
...

+

Milk Recording Data including MIR Spectrum

=

COMMON TRANSNATIONAL DATABASE

BUT !!

Because of differences between the instrumental responses of different MIR spectrometers, and because of changes in the instrumental response of a spectrometer over time, the use of calibration models developed at a certain time on a certain instrument with MIR spectra obtained on the same instrument after a period of time, or on another instrument, will usually lead to erroneous results.

Inconvenient to recalibrate instruments or may want to utilize a historical database.

Standardization procedures are needed

A decision needs to be made which instrument will be declared as **MASTER** instrument, the remaining instruments will be used as **SLAVE** instruments.

An own unique **standardization model for every master-slave** combination needs to be designed, describing the shift between the particular slave instrument and the master instrument

In order to be able to perform data standardization between the master and a slave instrument, an equal amount of measurements have to be done with both instruments.

New measurements will be later standardized using the standardization models built.

STANDARDIZATION APPROACHES

Find a transformation that maps the response of the slave instrument onto the master instrument

      Direct and piece-wise direct standardization

      …

Process the data from both instruments in a way that makes the differences disappear

      baselining and derivatizing

      multiplicative scatter correction, FIR filtering

      orthogonal signal correction

      prediction augmented classical least squares

      generalized least squares

      explicit deresolution

   …

PIECE-WISE DIRECT STANDARDIZATION (PDS)

Proposed by Wang et al.

This method transfers the MIR spectra from the instrument on which they were collected ('slave') to the instrument on which the calibration model was developed ('master').

PDS is based on the fact that the spectral information contained in a certain wavelength on the master instrument is highly correlated to the spectra of neighbor wavelengths on the slave instrument.

PIECE-WISE DIRECT STANDARDIZATION (PDS)

The PDS method is based on the fact that the spectral variation of spectroscopic data is limited to small regions. In PDS, the response r_1 of the standardization samples measured at wavelength j on the 'master' instrument is related to the wavelengths located in a small window (R_2) around j (neighbouring) measured on the 'slave' instrument:
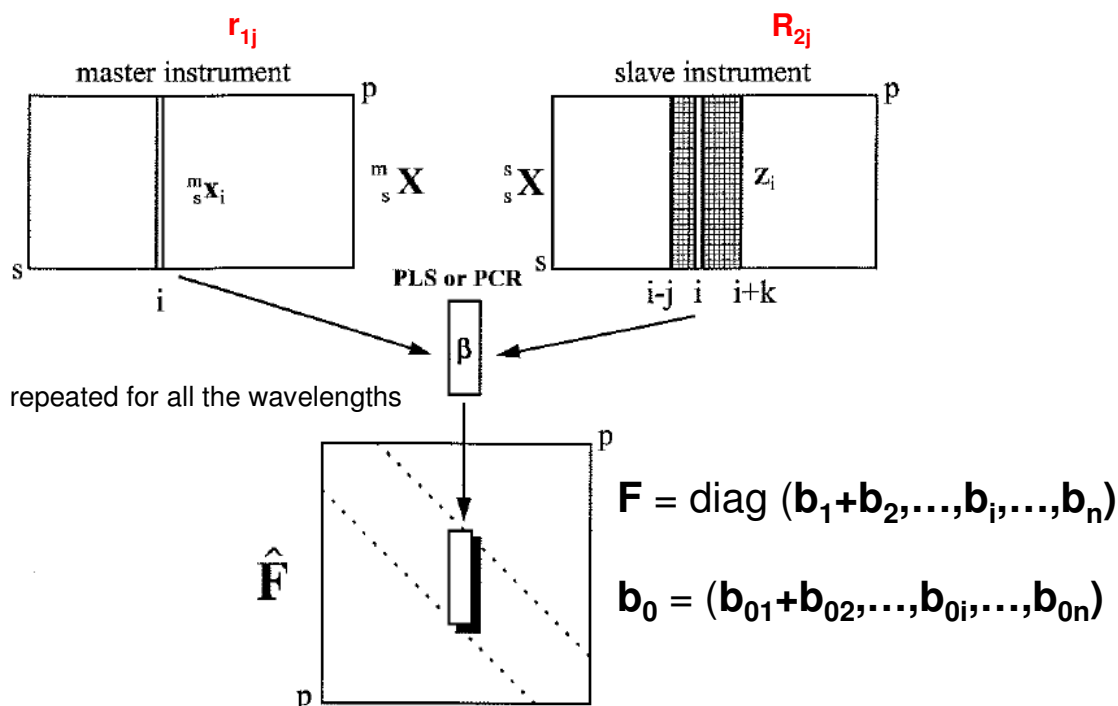

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk
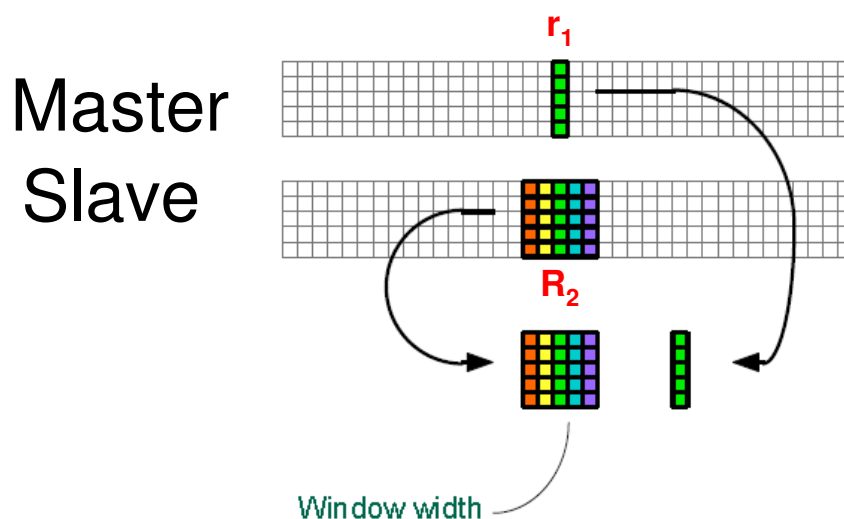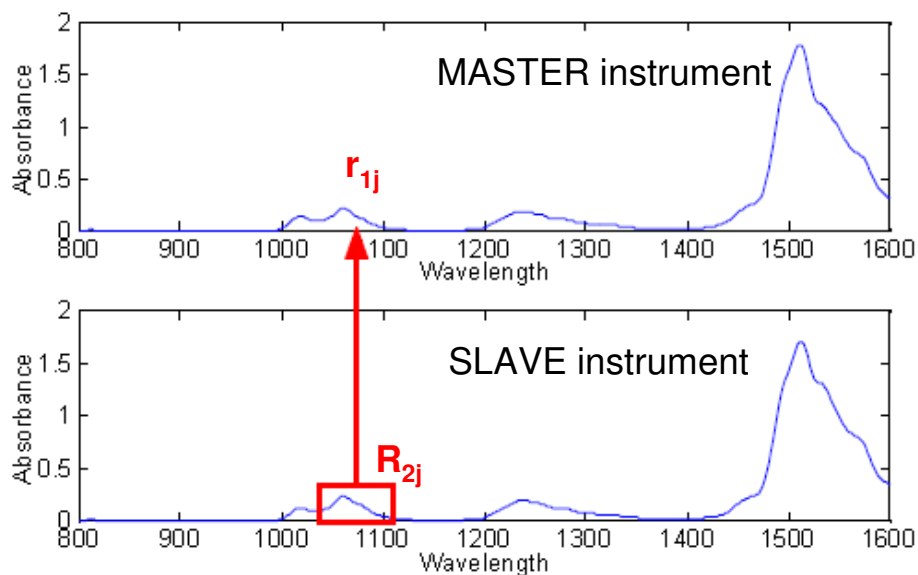
$$r_{1j} = R_{2j} b_j + b_{0j}$$

Where R_{2j} is the localized response matrix of the transfer samples and b_j is the vector of transformation coefficients for the jth wavelength and b_{0j} is the offset term.

The regression vectors (b) can be calculated by PCR or PLS method

$$F = diag (b_1+b_2,…,b_i,…,b_n)$$

$$b_0 = (b_{01}+b_{02},…,b_{0i},…,b_{0n})$$

Source: PhD thesis H. Swierenga, Univ. Wageningen, NL (2000)

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk

The F matrix and the b_0 vector are used to correct new spectra measured in the slave instrument, **$r_{2,unk}$**
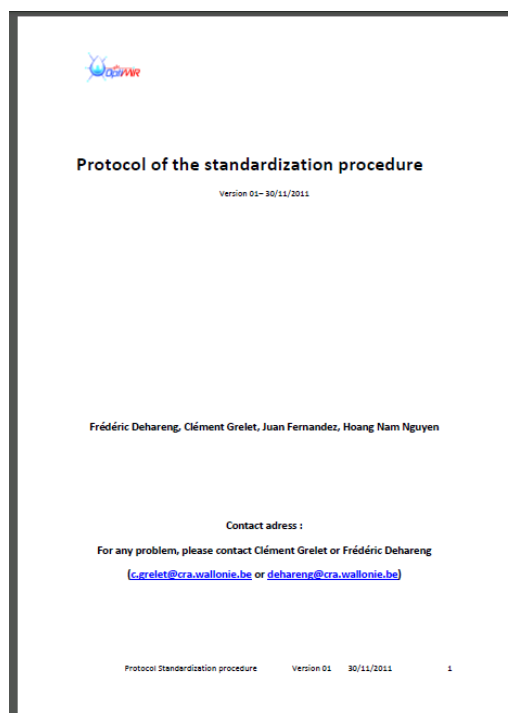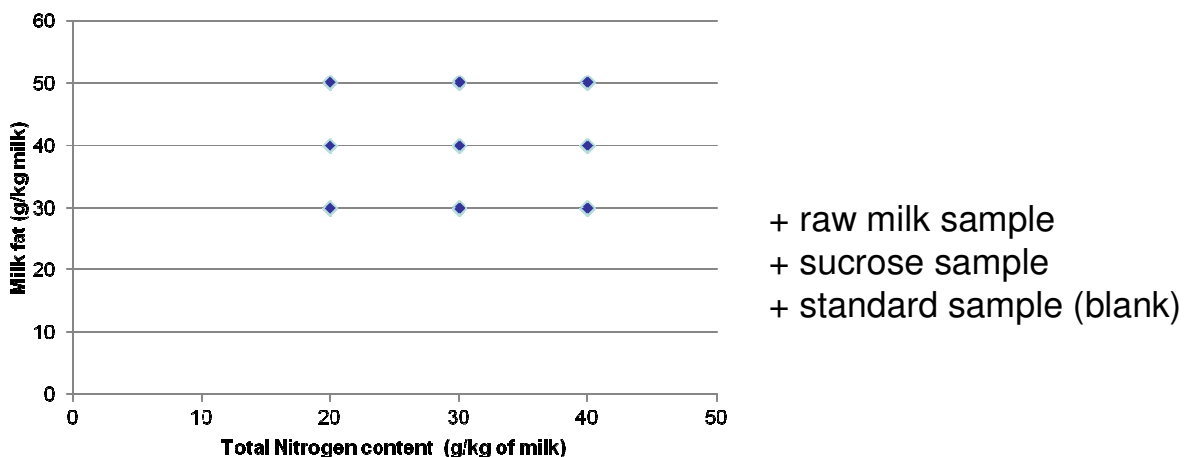
$$(R_{2,unk})_{std} = r_{2,unk}\ F + b_0$$

SOME RESULTS

OptiMIR Standardization of February – March 2012

➢25 laboratories

➢50 instruments

➢600 samples

Master FOSS (Battice Foss05009)

+ raw milk sample
+ sucrose sample
+ standard sample (blank)

STANDARDIZATION - MODEL

(February 2012)

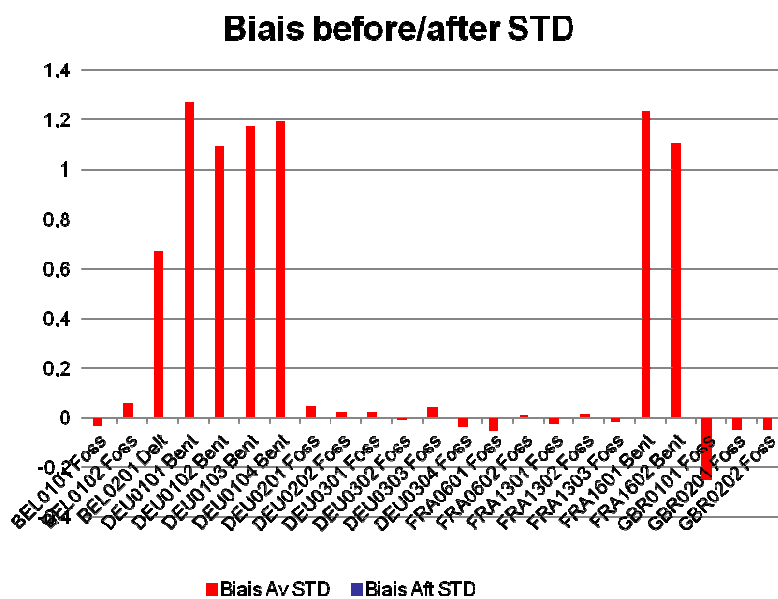|		BEFORE STD	AFTER STD
	Brand	Pente Master//Slave	P Master//Slave
BEL0101	Foss	1.002	1.001
BEL0102	Foss	0.976	1.001
BEL0201	Delta	0.799	0.983
DEU0101	Bentley	0.615	1.014
DEU0102	Bentley	0.670	1.016
DEU0103	Bentley	0.654	1.016
DEU0104	Bentley	0.642	1.015
DEU0201	Foss	0.991	1.002
DEU0202	Foss	0.989	0.997
DEU0301	Foss	1.001	0.998
DEU0302	Foss	1.004	0.998
DEU0303	Foss	0.988	0.998
DEU0304	Foss	0.989	1.001
FRA0601	Foss	1.018	0.997
FRA0602	Foss	0.996	0.996
FRA1301	Foss	1.007	0.996
FRA1302	Foss	1.001	0.997
FRA1303	Foss	1.038	0.996
FRA1601	Bentley	0.622	1.010
FRA1602	Bentley	0.659	1.005
GBR0101	Foss	1.064	0.995
GBR0201	Foss	1.007	0.996
GBR0202	Foss	1.005	0.994
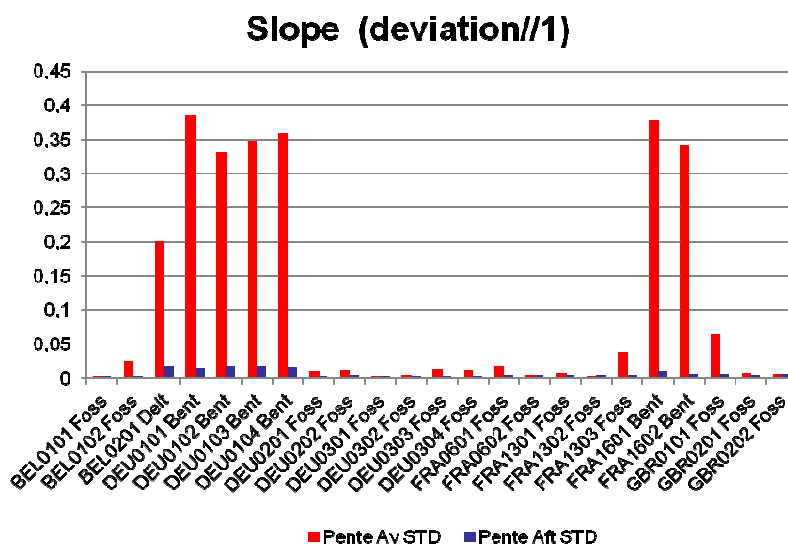
Master FOSS

STANDARDIZATION - MODEL

(February 2012)

	Brand	BEFORE STD Biais Master//Slave	AFTER STD Biais Master//Slave
BEL0101	Foss	-0.0324	0.0000
BEL0102	Foss	0.0583	0.0000
BEL0201	Delta	0.6689	0.0000
DEU0101	Bentley	1.2731	0.0000
DEU0102	Bentley	1.0893	0.0000
DEU0103	Bentley	1.1728	0.0000
DEU0104	Bentley	1.1917	0.0000
DEU0201	Foss	0.0466	0.0000
DEU0202	Foss	0.0217	0.0000
DEU0301	Foss	0.0185	0.0000
DEU0302	Foss	-0.0007	0.0000
DEU0303	Foss	0.0391	0.0000
DEU0304	Foss	-0.0332	0.0000
FRA0601	Foss	-0.0534	0.0000
FRA0602	Foss	0.0082	0.0000
FRA1301	Foss	-0.0232	0.0000
FRA1302	Foss	0.0144	0.0000
FRA1303	Foss	-0.0115	0.0000
FRA1601	Bentley	1.237	0.0000
FRA1602	Bentley	1.1067	0.0000
GBR0101	Foss	-0.2532	0.0000
GBR0201	Foss	-0.0496	0.0000
GBR0202	Foss	-0.0479	0.0000

Master FOSS

STANDARDIZATION - MODEL

(February 2012)

	Brand	BEFORE STD RMSE Master//slave	AFTER STD RMSE Master//slave
BEL0101	Foss	0.0331	0.0073
BEL0102	Foss	0.0648	0.0198
BEL0201	Delta	0.6908	0.0249
DEU0101	Bentley	1.315	0.0154
DEU0102	Bentley	1.1253	0.0152
DEU0103	Bentley	1.2096	0.0163
DEU0104	Bentley	1.2303	0.0168
DEU0201	Foss	0.0474	0.0074
DEU0202	Foss	0.0245	0.0055
DEU0301	Foss	0.0201	0.0078
DEU0302	Foss	0.0108	0.0098
DEU0303	Foss	0.0421	0.0117
DEU0304	Foss	0.0364	0.0117
FRA0601	Foss	0.0557	0.0047
FRA0602	Foss	0.0103	0.0064
FRA1301	Foss	0.0250	0.0076
FRA1302	Foss	0.0159	0.0064
FRA1303	Foss	0.1226	0.0272
FRA1601	Bentley	1.2788	0.0123
FRA1602	Bentley	1.1446	0.0073
GBR0101	Foss	0.2521	0.0097
GBR0201	Foss	0.0506	0.0084
GBR0202	Foss	0.0489	0.0108

Master FOSS

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk
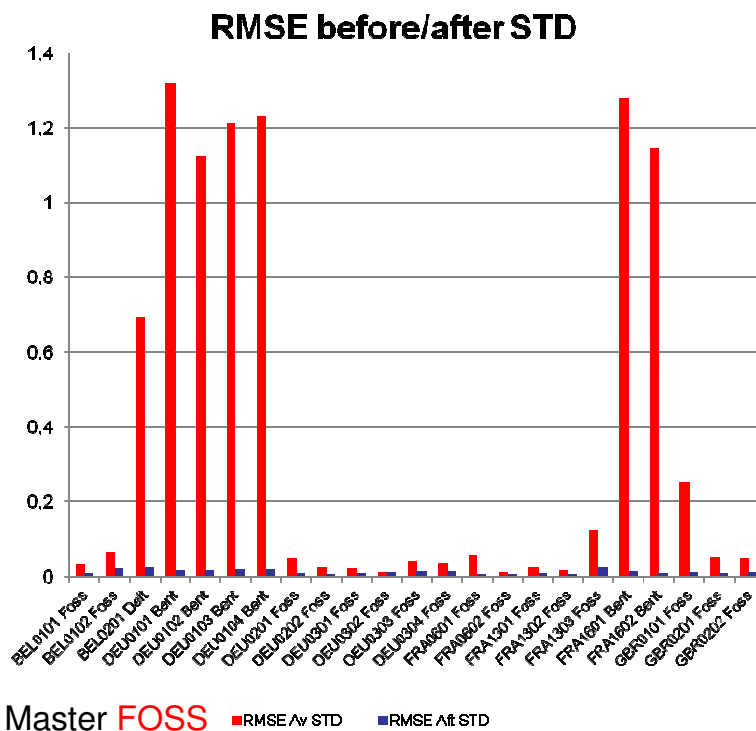
STANDARDIZATION - VALIDATION

(March 2012)

		BEFORE STD	AFTER STD
	Brand	RMSE Master//slave	RMSE Master//slave
BEL0101	Foss	0.0166	0.0226
BEL0102	Foss	0.067	0.0257
BEL0201	Delta	0.4864	0.0344
DEU0101	Bentley	0.9375	0.0614
DEU0102	Bentley	0.8107	0.0385
DEU0103	Bentley	0.8902	0.0395
DEU0104	Bentley	0.8957	0.0286
DEU0201	Foss	0.0116	0.0671
DEU0202	Foss	0.0288	0.0192
DEU0301	Foss	0.0778	0.0709
DEU0302	Foss	0.0652	60.029
DEU0303	Foss	0.0263	0.0182
DEU0304	Foss	0.0189	0.0505
FRA0601	Foss	0.0485	0.0095
FRA0602	Foss	0.0165	0.0126
FRA1601	Bentley	0.9205	0.0266
Mean		0.3324	0.0368

RMSE before/after STD
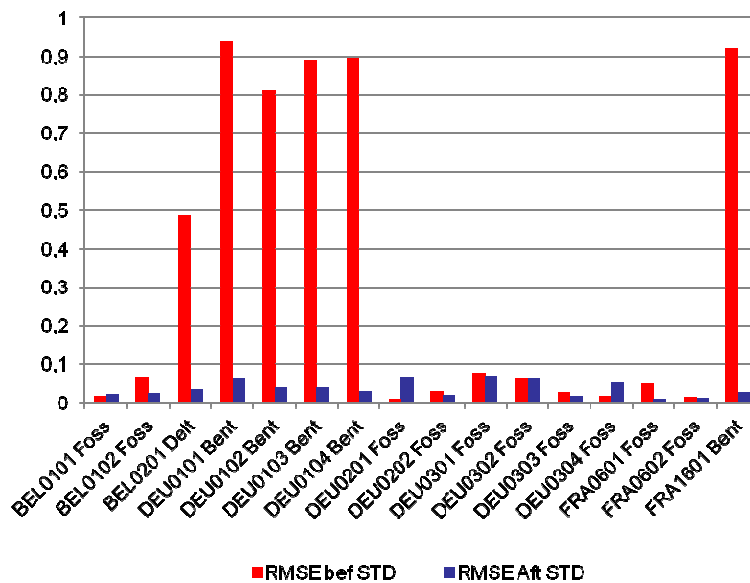
Master FOSS

STANDARDIZATION - EXAMPLE

Raw MIR spectra

Foss – Master 1060 wav.

Bentley – Slave 889 wav.

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk

STANDARDIZATION - EXAMPLE

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk
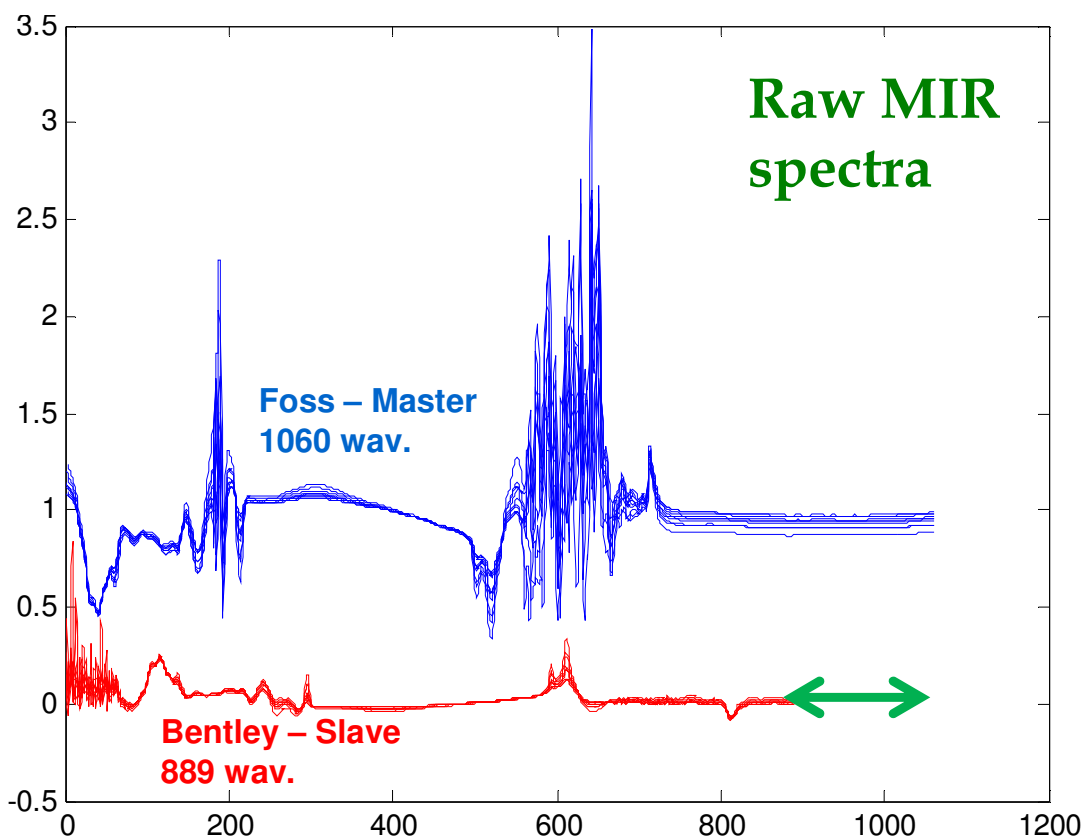
After standardization
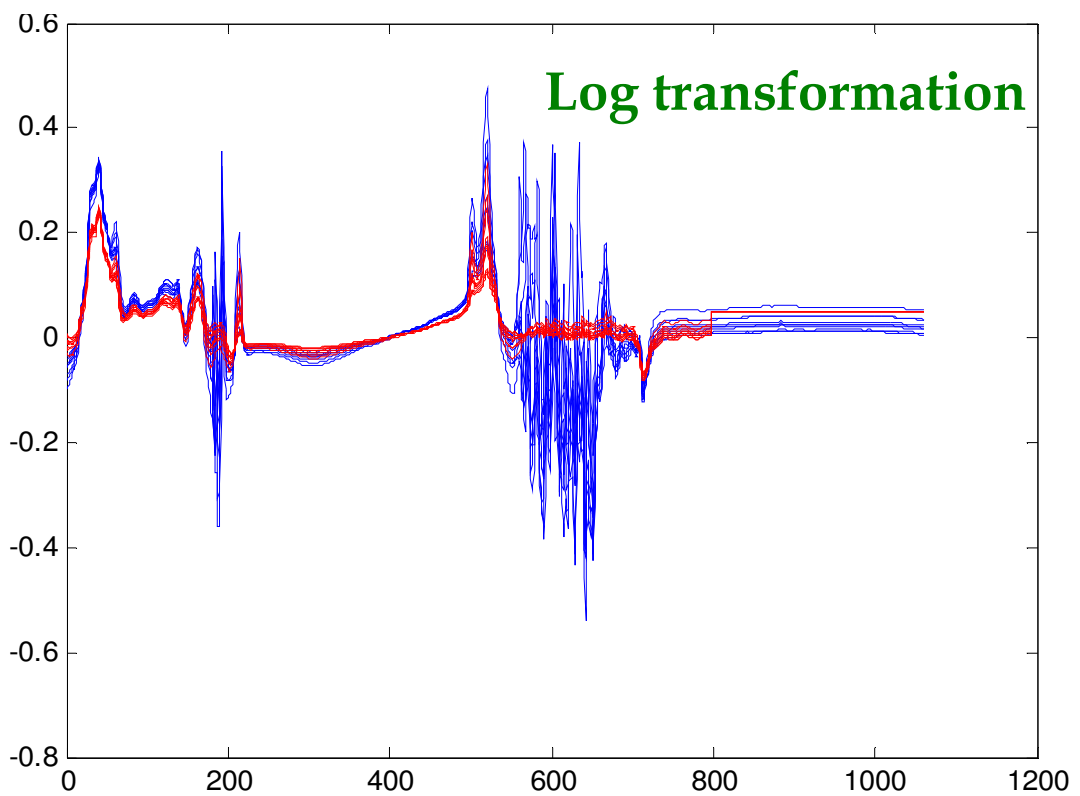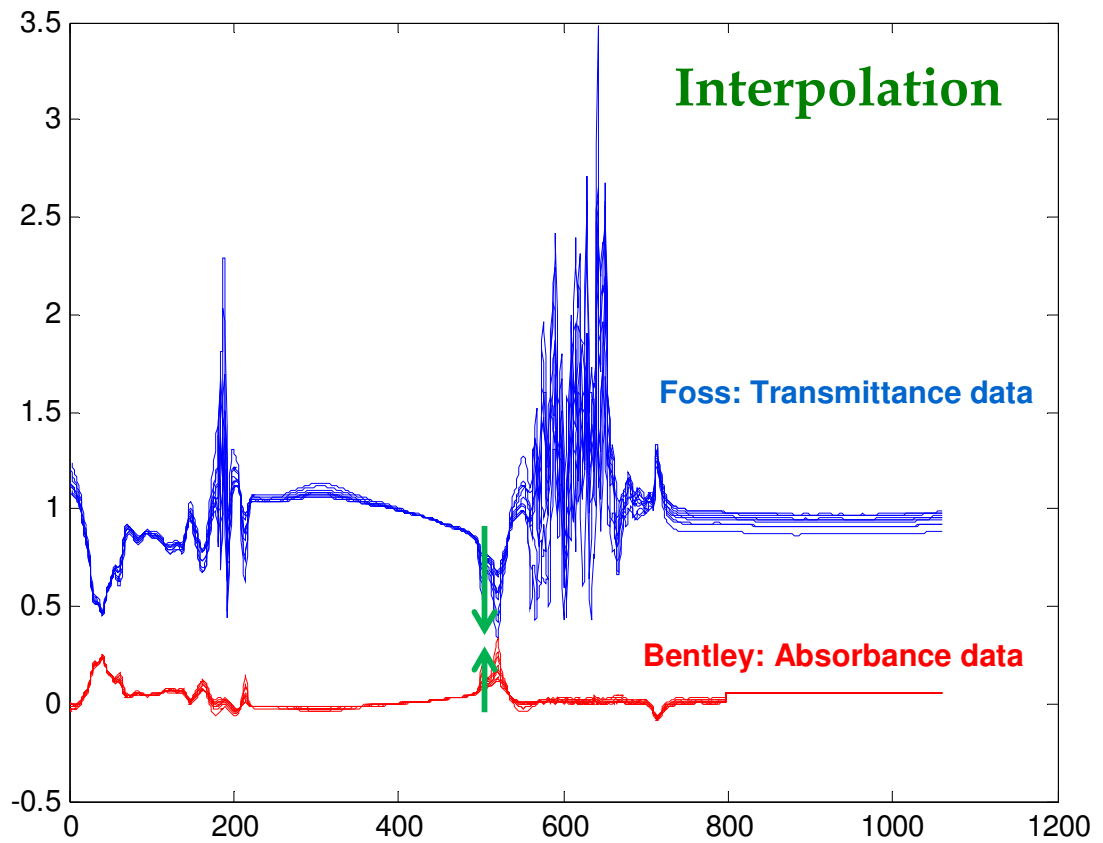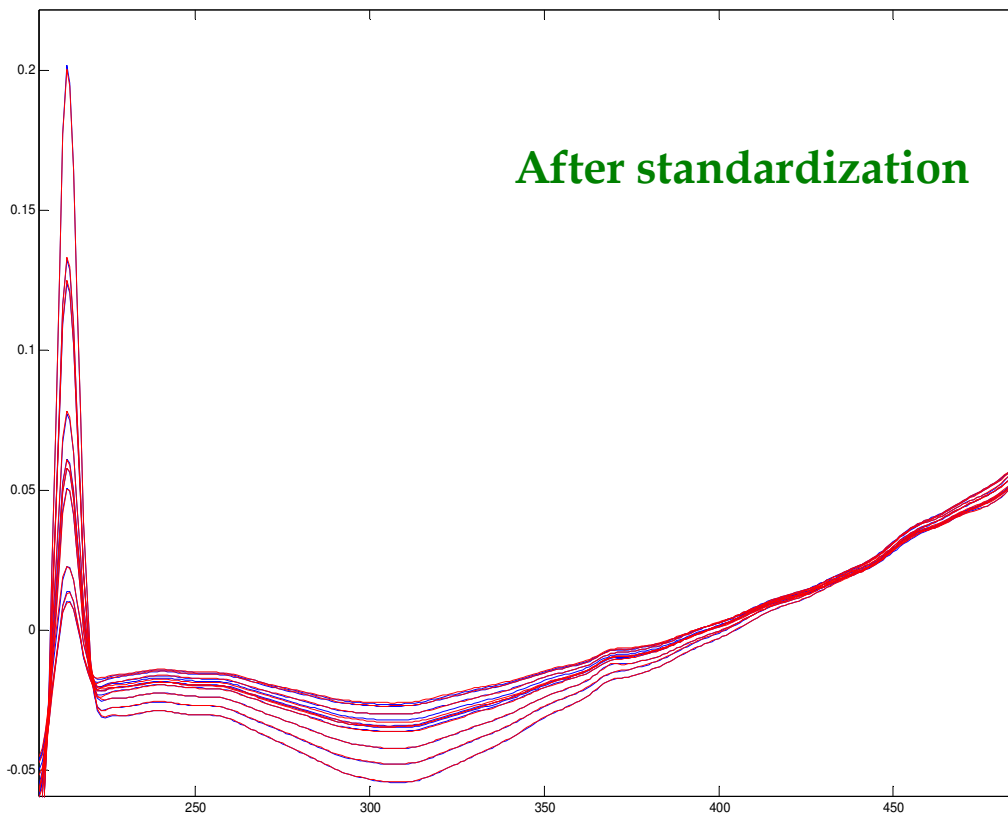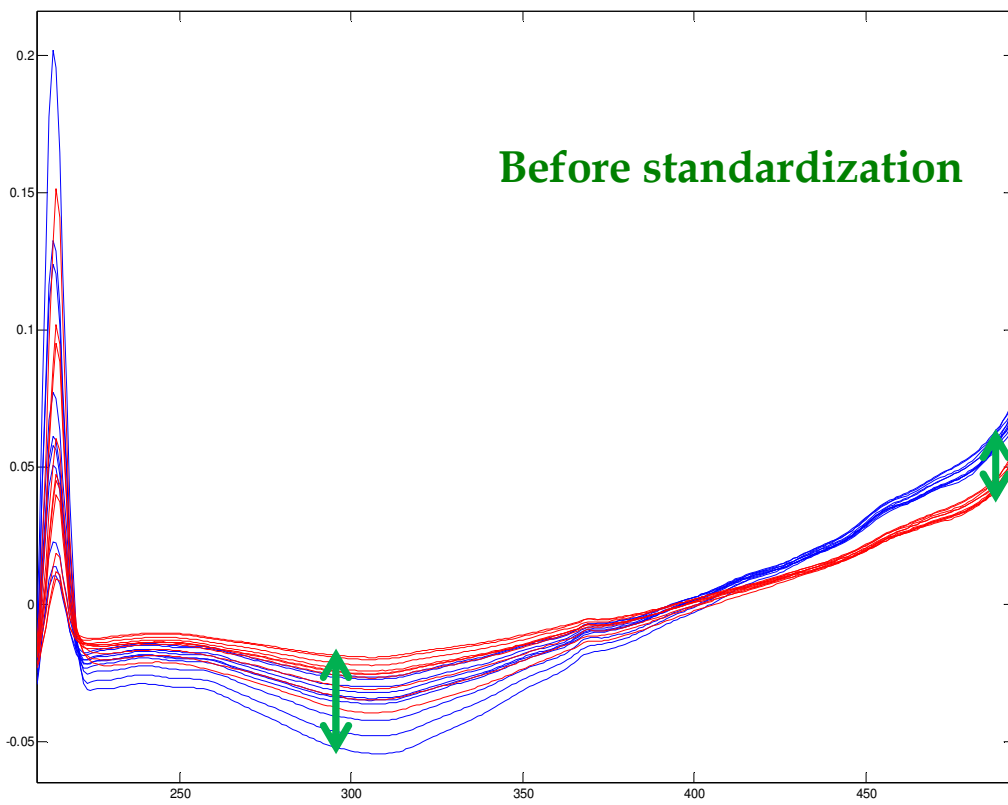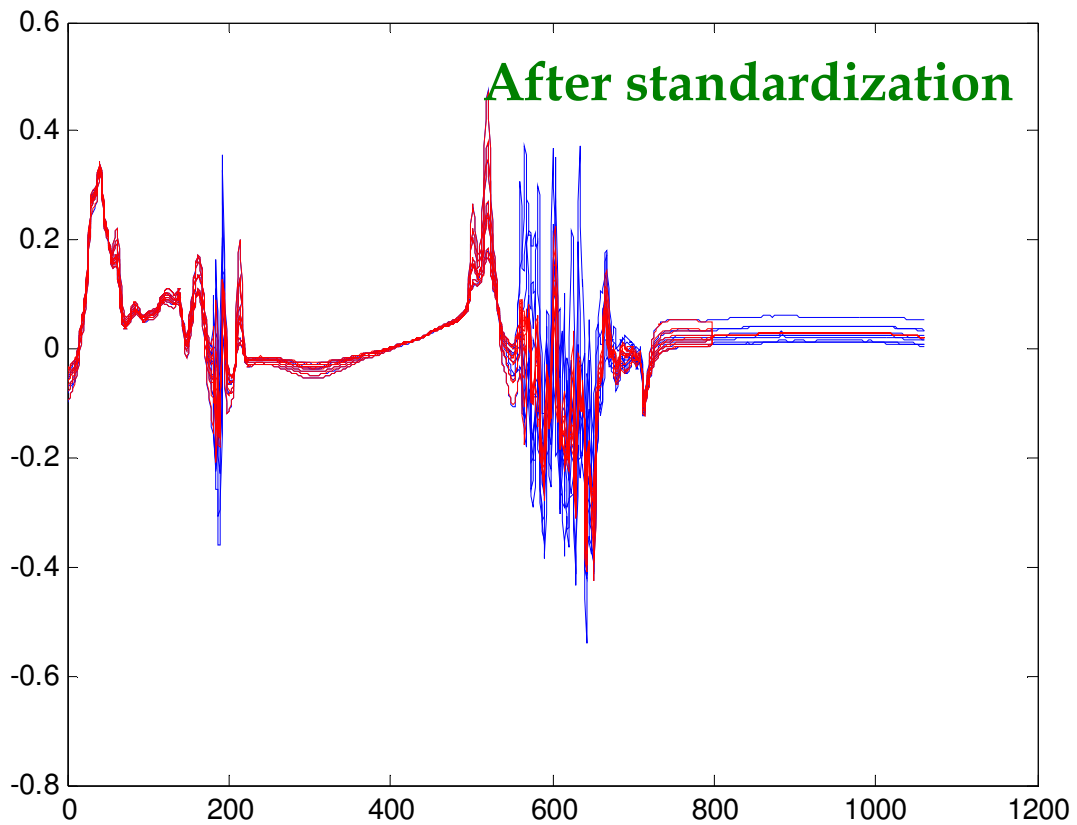
1236 spectra with reference values

Fat content equation

$R^2 = 0.997$
4 Latent Variables
RMSEC = 0.057579
RMSECV = 0.058406
Calibration Bias = -0.00031737
CV Bias = -0.00031853

Merging of spectral datasets from different MIR instruments used in the routine analysis of milk

Samples/Scores Plot of Xmgc,c & X2stdc,

DELTA slave
(CRA-W Delta)

RMSEP: 0.4715

R^2 = 0.996
4 Latent Variables
RMSEC = 0.057579
RMSECV = 0.058406
RMSEP = 0.47147
Calibration Bias = -0.00031737
CV Bias = -0.00031853
Prediction Bias = -0.43941

Samples/Scores Plot of Xmgc,c & yres,

DELTA slave
(CRA-W Delta)

RMSEP: 0.1650

R^2 = 0.997
4 Latent Variables
RMSEC = 0.057579
RMSECV = 0.058406
RMSEP = 0.16504
Calibration Bias = -0.00031737
CV Bias = -0.00031853
Prediction Bias = 0.14059

CONCLUSIONS

The standardization correction using PDS seems to work correctly during time for the different slave instruments available, including different brands.

The use of this standardization method is a crucial step for the OptiMIR project because it will allow the use of only one single equation by property for all the different instruments.

Useful to pool the resources of milk recording organizations and research centres and MIR milk spectra to be used as indicator of the cows' status.
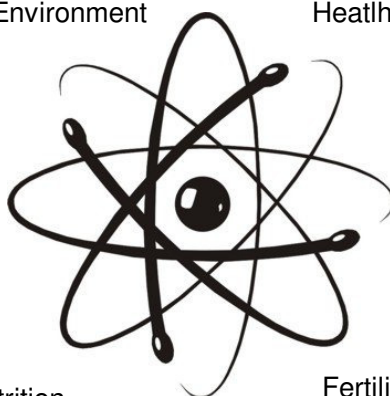
Discussion and conclusion

The following is a summary of the main pieces of successive discussions either just after each presentation or at the end of each half session. It was established from taken brief notes and related talk memories and reformulated for better understanding with appropriate context recalls.

Olivier Leray, FGE-ICAR, Poligny, France
Harrie van den Bijgaart, Qlip, Zutphen, The Netherlands

Part 1:

<u>1- ICAR Reference Laboratory Network – Objectives & stage of progress (O Leray):</u>

ICAR Reference Laboratory Network is used as a vehicle to broadcast and help to implement harmonised analytical practices worldwide in the ICAR sphere. It was shown that not all ICAR members organisations involved in dairy production had adhered to the system by appointing a reference laboratory. As well it was demonstrated how important the role of these laboratories can be in anchoring local routine laboratories to a universal reference and providing indication on the true uncertainty of their results. It is reminded that comparable equivalent results are a strategic point in milk recording with regard to genetic evaluation and trade.

The actual issue is nowadays on how to increase the participation of ICAR MOs in the reference laboratory network and provide them access to the international reference. The chairman explained that at first in 1994 it was a volunteer demarch proposed to organisations to enter an analytical quality assurance system and accompany a general QA trend made to harmonize quality levels and face the upcoming globalization of trade. Progressive implementation and development expansion was expected throughout time through repeated promotion and communication through conferences like today. This has been the normal upgoing dynamic observed untill a plateau was reached in 2003 that is needed to pass through.

The question arose about the fact that this is no more the only original QA interest of individual MOs but the ICAR collective interest which is addressed. Hence one is wondering whether a clearer and more ostentatious step is required possibly by making participation mandatory. Such an option is felt well in line with the ICAR QA policy developed with the ICAR audit system and delivery of a Certificate of Quality. This is retained as a further proposal to ICAR Board.

<u>2- International survey on somatic cell counting – Situation of lab network organisation and practices (S Orlandini) / 3- PT scheme interlinkage and international laboratory anchorage (O Leray)</u> :

The joint IDF-ICAR project on Reference System for Somatic Cell Counting derives from the ICAR international interlaboratory experience. It is to describe and organize in an optimal way an interlaboratory activity in order to assess laboratories and proficiency testing schemes, characterise reference materials and determine international reference values. A final goal is to distribute trustworthy reference values through international standard reference materials.

A survey done by the joint IDF/ICAR working group allows results permitted having a global view on existing regional laboratory networks or PT schemes and the established cross-linkages and international anchorage (ICAR) worldwide. It is proposed that those replies taking part in the questionnaire (>200 replies!) on somatic cell counting should also be encouraged/invited to participate as new participants in an international interlinked system. Motivate them to do so!

It is also noted that tools have been developed for implementing a reference system based on traceable competence of participating laboratories. But getting them implemented is not such easy and will take quite some efforts from all parties involved. Indeed PT scheme bridging and international anchorage have

been for long only theoretical and were concretized only from 2010 using the model here presented with taking example of somatic cell counting although it is general and valid for all methods.

At the present stage the laboratory network for SCC is only virtual based on existing regional networks and interlinkage. Actually it is to be implemented but few laboratories practice regularly the reference methods (DMSCC). Regular lab training and performance evaluation in DMSCC and in-house calibration in automated SCC is to be organized. For all the needed interlinking there is still a considerable and indispensable role for secondary reference materials.

It is mentioned that handling, transport and storage can influence/affect strongly the quality and the durability of reference materials and that these various dimensions must be under control. Reference materials should meet criteria as listed in ISO Guide (RMs Series 30 to 35). Additional guidance is in a document being finalized by the Project Group. Transportation is covered therein. There are objective tools available to assess the suitability of applying procedures for handling, transport and storage. Suitable means of preservation seem to be in the application of preservating agents, deep-freezing and/or lyophilizaton of reference material. However, all these options bear to some degree of risk. These risks will be outweighed. Special attention is given to custom obstacles and the need for a meticulous preparation of shipment and accompanying documents. Identification and prior contact with key services / persons in countries may be needed in some countries.

Being asked for further clarification on the presented PT scheme interlinkages, the speaker stresses that the real picture was presented. Many interlinkages can be identified and provide a robust basis for further intensification.

More knowledge is acquired about reference materials for somatic cell counting and clearly the future will be with improved practices and results. Trust and objectiveness are needed around labs. No one is willing to deviate so there is a need to define the system to assist the lab and avoid discrepancy and achieve compliance. In that frame also ICAR Board is thinking about more stringency, this to be able to trace compliance in practice.

Participants were asked on their interest in taking part in a reference system approach. US experience was shared: 4 labs participate in national proficiency testing, partly linking up to an international proficiency testing scheme. It is well agreed with the principle of a reference system approach and the striving to obtain better equivalence. But the difficulty is in how to bring this all closer together. Who is going to say you are out?
Answers given were that this is the aim of the project to implement an unique worldwide accepted source of true values (consensus) with growing the whole project together and seeking authorative approval and coordination. The application of the interlinkage / anchorage to SCC as presented is an example of how it can work.

As a conclusion the need is expressed for a concrete structure to handle these issues. That brings a clear assignment for the follow-up work to do. This was not further contested.

<u>4- ICAR Inter Laboratory Proficiency Testing – Ireland Update - Review 2002-2011 (M Burke) :</u>

An update is given on the milk recording analysis system which is run in Ireland and its integration in the ICAR AQA system. Ireland joined the ICAR system in 1996 and nominated Teagasc Moorepark to the ICAR reference laboratory network.
The Irish DHI milk analysis system is organised with four dairy laboratories anchored to the Technical Services Laboratory of Teagasc MoorePark. The latter has implemented a centralized calibration through reference materials for MIR milk analyzers and somatic cell counting. Beside regular proficiency testing trials are run. Labs are characterized through three colours white (correct), yellow and red according to three ranges and occurring biases. Like in soccer, yellow means a warning and is asking for attention, a red card will result in serious demand for a real extra effort so to comply again. Statistical compliance evaluations are made on occurrence frequencies of both colours.

Referring to the former presentation it is recognized that the used reference values can be biased. However this can only be within accepted limits of error thanks to applied QA and anchorage to the ICAR ref lab network. Reference values are produced by Teagasc Moorepark with reference methods.

The speaker mentions that sampling error is a renewed critical issue especially with regard to the carry-over effect between successively milked animals. For animal health monitoring through milk recording samples, carry-over may be even more relevant.

Part 2:

5- Determination of milk protein composition by mid infrared spectrometry (M Ferrand) :

Individual protein composition is of a great interest for nutritional, functional, and technological issues, similarly as fatty acid compositions hence determining possible levers to influence protein composition at the farm level is particularly important. High throughput phenotyping methods are sought through and FTMIR is foreseen as a first attempt.

The reference method applied was LC-MS, thereby identifying at least 25 different molecules. Details can be found in the publication of Miranda et al. Concerning the statistical techniques there were several alternatives evaluated like the Penalization Method, Ridge regression, LASSO and Elastic Net. However, that did not bring any improvement as compared to PLS.

Critical points with the application are a robust sample set with accurate reference method results, gain in accuracy by counteracting proteolysis and a harmonization system between labs. Harmonization system can be the same as for fatty acid composition in milk in the Phénofinlait programme as presented in Bourg-en-Bresse (FR) in 2011.

To a question on the actual value for breeding in general beside the individual cow level, the speaker answered that value was for both, but she can't get in more detail. At present, the protein FTMIR project has not yet had a follow-up in France.

6- Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (M Cassandro):

Much interest is given to predict directly technological parameters on individual animal milk with high throughput mid infrared analysis and open the door to genetic evaluation on cheese making ability. FTMIR is used to bypass the determination of different parameters usually measured and combined separately. The goal is to obtain directly the key useful information needed for the targeted purpose.

Possible correlations between coagulation properties of the milk and other traits were not evaluated yet. Nevertheless there is indeed a significant correlation with the casein content (itself tightly correlated with cheese yield) but in the model there are more factors of interest involved to enable predicting milk clotting characteristics at cheese making. A Foss-supplied calibration model for the MilkoScan FT6000 is applied to extract spectral data in routine.

7- Merging of spectral datasets from different MIR instruments (J Fernandez):

Available spectral mid infrared information is getting larger and larger with technology improvements. The ranges of possible parameters prediction and the sophistication of new parameters or investigated information are increasing thus making utilisation spread over larger scales not restricted to the interest of a sole laboratory but to all the laboratories of which results may have to be compared. The predicited characteristics are getting more qualitative (beside quantitative determination) to move forward to expert systems and have access to immediate decision with the associated statistical risk of error. This induces costs significantly higher to establish the reference than in classical laboratory analysis since the latter more often results from expensive surveys and expert diagnoses. New strategies are to develop collaborative programmes involving several partners and laboratories where the huge work load can be suitably shared as well as the outcomes. The core tools to build such a system are spectral data bases

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which then can be used to create new analytical tools for the sake of all the partners. This is the case for the Phénofinlait programme in France and Optimir and RobustMilk programmes at the European scale undertaken from 2008.

Since there are different devices and devices brands / manufacturers one major issue lies in establishing an exact correspondence between mid infrared spectra of each FTMIR spectrophotometer type and brand so as to obtain identical predictions whatever the device and location with the same milk sample. Such a task has been undertaken since 2008 and subsequent progress from different sources was presented last year in Bourg-en Bresse (FR) 2011. Last developments were since made since then in the Optmir programme as here presented.

As a first remark the chair appreciated that the standardization and merging of spectral datasets had seemingly found a solution. However a subsidiary remaining issue will be the harmonization of databases for merging and consolidation where different spectral references are used. The speaker indicated that these are first results obtained only in fat prediction and next confirmation should be made for more sensitive milk components such as fatty acids.

About changes brought in the instruments the speaker mentioned a quite stable behaviour of FTMIR instruments in normal conditions but upon repairs or servicing there is a necessity to check and re-estabilish the equivalence of the spectrum again as normal.

The question was raised on the absoluteness of the master instrument as reference provider, i.e. to which golden reference standard (re reference spectrum) is it anchored. The possibility to use deep frozen milk reference materials to standardize instrument spectra and allow long term preservation and wide broadcasting was mentioned. The positive outcomes of Phénofinlait programme in spectrum and data harmonisation using deep frozen milk samples and orthogonal fat, protein, lactose arrangement were mentioned (ICAR 2011) thus indicating the way yet opened. Similar experimental milk sample set design is used in Optimir.

The discussion came to the manufacturer standard solution (Equalyzer, Foss) and its equal validity for qualitative and quantitative analytical approaches that was confirmed by the speaker and Foss attending representatives.
The chair reminded the utmost influence of homogenization quality on biases between instruments based on the observations made for fatty acids in the Phénofinlait programme with Milkoscan FT6000 prior standardized with the Equalizer solution. He emphasized on that any reference material should take into account the homogenization effect by having a fat globules suspension able to react similarly to milk fat. He noted that using a milk posed the problem of the the stock and reproducibility (milk is variable) and that the optimum would be a chemically reconstituted solution with standard fat emulsified phase that can be exactly reproduced at each preparation. This would serve to align all the spectrophotyometers answers. Thereafter a new issue for scientists and standardizers should be to define the standard spectrum target.

Question was then raised about the spectra alignment with Bentley devices and whether or not a standard solution similar as Equalizer is used. Bentley representative indicated there is no such solution used since instruments are very stable and regular checks prior running analysis are made with water.

In the end the potentiality of orthogonally designed sample sets already used for fat, protein and lactose in mid infrared calibration (IDF 141:2000) is pointed out for fatty acids and individual protein with regard to large range coverage and compensation of eventual problems in intercorrection. It is anticipated that good accuracy can be obtained from robust global calibrations.

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Conclusion

Globalization by bringing significant changes in society and economy results in new challenges for the dairy analytical sector and new strategies for international organisations such as ICAR.

The presentations gave examples of new (innovating) approaches to handle analytics at a broader level in order to assure analytical harmonisation and equivalence worldwide. These on-going developments are processed within a network of partner experts and organisations and thanks to regular information in similar conferences and meetings in ICAR and IDF. A number of international experts are attending and will relay relevant requests, suggestions and ideas to other workshop such the next ICAR analytical brainstorming workshop and technical bodies (e.g. ICAR and IDF-ISO working groups) for the sake of the dairy community.

In this way the chairman Olivier Leray invited the participants to join the brainstorming workshop scheduled the next day so as to continue and refine the thinking in a still more interactive session and indicate that some reporting can be made the next week at the IDF-ISO Analytical Week 2012 in Tel-Aviv.

To conclude the chairman warmly thanked the speakers and attendees and invited them all to attend the next conference meeting of ICAR Reference Laboratory Network in Berlin during the next Biennial ICAR Session 2014. Christian Baumgartner (organising committee) provided some information on the event and pointed out that there will be a joint organisation of ICAR session and IDF-ISO Analytical Week 2014 in succession with an overlapping part common to ICAR and IDF for persons with shared interest. He expressed his hope and wish that it could meet the launching of the IDF-ICAR SCC Reference System.