"International Strategies and New Developments in Milk Analysis"

Fifth ICAR Reference Laboratory Network Meeting

Riga, Latvia, 1st June 2010

MA SC
ICAR Sub-Committee
on Milk Analysis

FOREWORD

ICAR Reference Laboratory Network is now in existence for fourteen 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 was identified as the end of the first period of the implementation/development of the AQA system of ICAR. From Kuopio, it was decided to produce practical guidance and tools in order to facilitate the work of reference and routine laboratories and harmonise practices in ICAR countries. That decision has directed the choice of the programme in Niagara Falls (USA) in June 2008 as focused on the practicability and recommendations for international traceability, anchorage, reference systems, centralized calibration, analytical equivalence while, that year 2010, in Riga (Latvia) the focus is made, on one hand, on the efficiency of ICAR analytical strategy and the actual developments reference systems as a follow up of Niagara Falls and, on the other hand, on new analytical developments of interest for milk recording including also an example of herd management through on-farm milk analysis.

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

Poligny, 13 September 2010

Olivier Leray
Chair of ICAR Sub-Committee on Milk Analysis

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International Strategies and New Developments in Milk Analysis

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Döring	Franziska	LKV Sachsen-Anhalt e.V.	56	Germany
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Last name	First name	Organisation	Reg.	Country
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Hammel	Manfred	LKV-Brandenburg	38	Germany
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High	Jere	Lancaster DHIA	10	United States
Hoefelmayr	Tilman	WMB AG	05	Switzerland
Huquet	Bérénice	Institut de l'Elevage	61	France
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Karakoyunlu	Ibrahim	Cattle Breeder's Association of Turkey	17	Turkey
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Koka	Helle	Estonian Animal Recording Centre	33	Estonia
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Koltsidas	Nikolaos	Genetic Identification	16	Greece
Kulesza	Bogdan	Polish Federation of Cattle Breeders and Milk Producers	09	Poland
Kuresoo	Mart	Estonian Animal Recording Centre	31	Estonia
Lama	Jorge	Cooprinsem	44	Chile
Lefebvre	Daniel	Valacta	30	Canada
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Mészáros	Gyula	Livestock Performance Testing Ltd	48	Hungary
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Ozollapa	Inese	Institute of Food Safety, Animal Health and Environment BIOR	53	Latvia
Park	Sang Chool	Korea Animal Improvement Association	59	Korea

Last name	First name	Organisation	Reg.	Country
Pinsky	Niv	S.A.E. Afikim	45	Israel
Püttsepp	Veera	Estonian Animal Recording Centre	32	Estonia
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Sales	Henrique	Direcção Geral de Veterinaria	67	Portugal
Saunier	David	France Conseil Elevage	04	France
Savickis	Saulius	State Laboratory Pieno Tyrimai	64	Lithuania
Schallerl	Franz	ZAR	49	Austria
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Skopane	Lana	SIA Piensaimnieku Laboratorija	12	Latvia
Szatmári	Lídia	Livestock Performance Testing Ltd	46	Hungary
Szewczyk	Agnieszka	Laboratorium Oceny Mleka (KCHZ)	08	Poland
Tapki	Ibrahim	Namik Kemal University	21	Turkey
Trejo	Carlos	Cooprinsem	45	Chile
Urbsiene	Laima	State Laboratory Pieno Tyrimai	63	Lithuania

Opening and introduction

Christian Baumgartner (Session Chair)
Milchprüfring Bayern e.V., Wolnzach, Germany

opening remarks

- ICAR Reference Laboratory Network is a forum for all analytical people within ICAR.
- Lab work is an essential part of DHI work!
- QA is crucial for successful (lab) work.
- ICAR Quality Certificate is an essential step forward to world wide equivalence of procedures and results.
- Ref Lab Network will give support and structure to all kind of efforts within ICAR to safeguard equivalence in the analytical field.

opening remarks

- This year´s session offers two blocks of information:
  - new developments and advances in building up reference systems and
  - new parameters and methods to add value to the DHI business.
- Be open to receive this information and don´t hesitate to discuss with us (your colleagues!) all matters of interest and/or concern!
  Exploit and utilize this session for yourself!

International Strategies and New Developments in Milk Analysis

Part 1: Reference systems – New developments

ICAR Reference Laboratory Network - Objectives & Stage of Progress in 2010

Olivier Leray

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Abstract

A policy for quality assurance on milk recording analysis has been developed from sixteen years by ICAR according to the orientation proposed in Ottawa in 1994. So called analytical quality assurance system has been based on the adoption and use by laboratories of same technical guidelines produced by ICAR and a structuring model based on national networks for laboratory monitoring at country levels and an international network of reference laboratories. The latter network is the corner stone of the system as it allows to anchor countries to common reference values defined internationally through proficiency testing programmes and offer a adequate framework to characterize reference materials well made to distribute analytical trueness. The efficiency of the system, as depending on general recognition, requires the largest participation of member organisation of ICAR.

1. History

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

That policy was implemented and handled by the Working Group on Milk Testing Laboratories of ICAR until 2006. then was continued by the enlarged permanent working party, the Sub-Committee on Milk Analysis.

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

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

2. Structure and architecture

The network is built on a hierarchical centralized model for the sake of vertical forth and back communication for harmonisation and provision of standard technical information and offers of services from the coordinating committee, the ICAR MA SC. Horizontal communication and collaboration between laboratories is encouraged and made possible thanks to a member list regularly updated. This is an organisation in two (possibly three) levels of network implementation as national (or regional) and international.

A third layer can exist for instance in federal countries where as well regions can organise labs in network or be a prospective challenge for the future to monitore on-farm analysis from regional laboratories.

2.1 National level:

National networks gather the milk recording laboratories of the country (or the organisation) and establish a national (or local) coordination based on international standards and guidelines, Good Laboratory

Practices, as mentioned in the ICAR guidelines. It is expected the coordination is made by a laboratory with high competence in every aspects of milk analysis, so-called “reference laboratory”, so as to be able to run the missions needed to assure analytical quality. This is to monitor routine laboratories, teach and traine lab technicians, evalutate and implement new techniques, methods or instruments, advise laboratories as well as milk recording organisations by which it is commissioned.

The reference laboratory is also requested to establish concrete tools to assess laboratory performances so as to assure confidence nationally to stakeholders. This is mostly attained by the organisation of national proficiency testing schemes. Beside the provision of technical tools in the form of reference materials to check reference methods or calibrate routine methods is highly recommended by ICAR for the ease in the laboratory work and analytical result security every day.

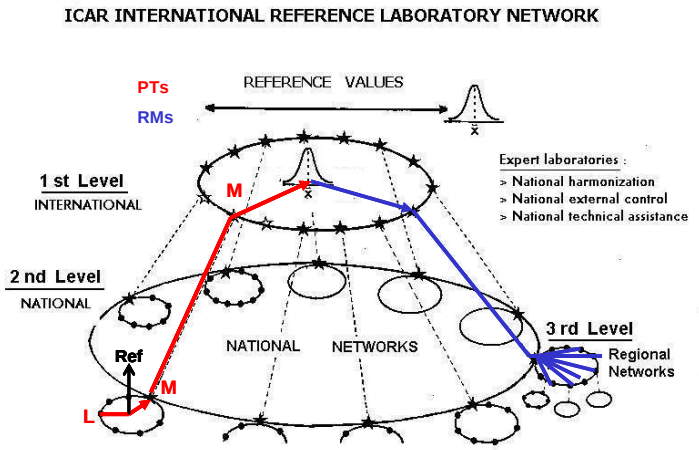

Figure 1. Structure of ICAR networking model with bottom-to-top/top-to-bottom circulation of information

2.2 International level:

The reference laboratories are invited in an international coordination by ICAR and can become member of the ICAR Reference Laboratory Network. This is made through the nomination of the national ICAR member organisation and under the condition the candidate laboratory and the national organisation adopt the model of functioning, as far as the local situation permits so, and comply to the ICAR guidelines. Such reference laboratories may have been existing for other purposes prior to the ICAR Reference Laboratory Network implementation but however in a number of case the reference laboratory must have been created and competence acquired so as to cover the largest panel as promoted by ICAR.

3. Roles

3.1 Analytical traceability and anchorage

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

The national reference laboratories operate as bridges to transmit the precision traceability from the international level to national levels thanks to interlaboratory studies carried out regularly at both national and international levels. Interlaboratory studies allow to measure laboratory bias to the reference laboratory which relays to the international absolute reference through its own bias to the international

reference values. Elements of trials reports allow laboratories to calculate the uncertainty related to its practice with the method.

Beside every member of the reference laboratory network can be invited to participate in international collaborative studies to characterize certified reference materials (golden standard) and establish reference values for every reference laboratory of the network therefore can contribute to provide tools to routine laboratories to measure the trueness of results and perform adjustment according to their needs.

3.2 Interlaboratory proficiency studies

Since 1996 an annual interlaboratory proficiency scheme has been regularly run twice a year for methods used as reference to calibrate routine methods for fat, protein and lactose in cow milk. It was complemented from 1999 with methods for methods for urea and somatic cell counting. From 2009 participant number has significantly decreased and in the first round of 2010 it is 15 for fat, 16 for protein, 14 for lactose, 13 for urea and 16 for SCC.

However significant improvement of analytical performances was noted throughout years and today the overall precision observed within the network appears fit to standard precision values stated in respective international method standards.

4. Membership

Any laboratory commissioned to monitor routine testing laboratories should be invited by their national organisation to join the network. Competence and expertise requested as eligibility criteria to belong to the network are one or more of the followings :

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

and the ideal situation is where the reference laboratory covers every competence item and therefore can ensure consistency and continuity in missions to routine laboratories. In some situation competence and expertise may be in several laboratories which may allows more laboratories per country.

For specific situation where only few laboratories with no national co-ordination, individual routine laboratories may also join the network so as to benefit to a direct anchorage to the international level whereas, in well structured local situations, so-called reference laboratories can establish the junction between routine labs and the international level.

5. Evolution and stage of progress

The numbers of laboratories qualified for various scientific/technical mission have increased gradually from 1996 to 2003 and moved to stabilisation attained in 2007 (Figure 2). In mid 2010 there are 38 of 32 countries involved in cow milk analysis, of which as well 17 work for goat milk and 14 for sheep milk.

Table 1. Worldwide representative-ness and of member number per country in 2010

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

(n) : number of member(s)

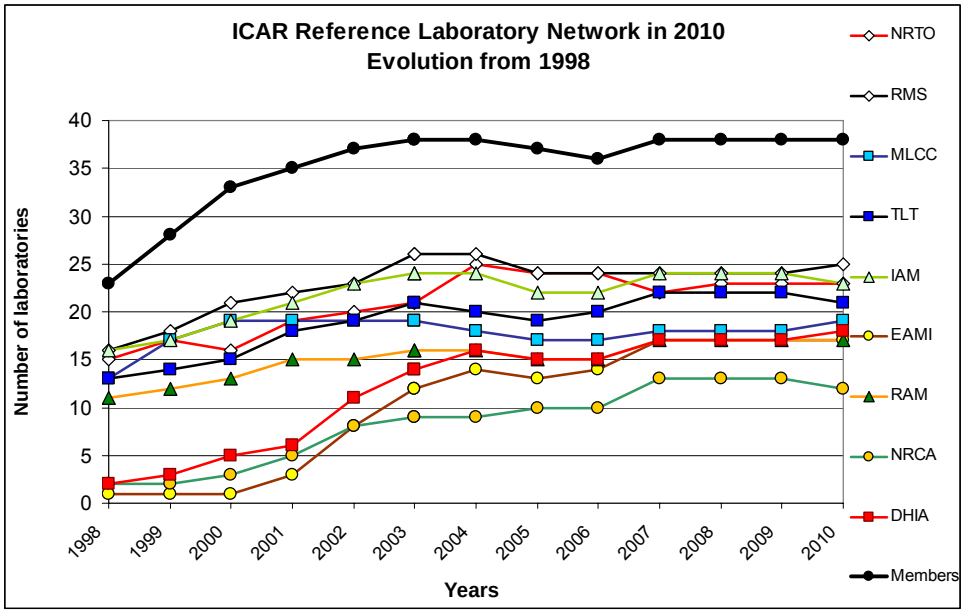

Figure 2. Evolution of membership and expertise in ICAR Reference Laboratory Network from 1998 to 2010

In 2010, with regard to the number of eligibility criteria declared by laboratories 75% of competence items are realised by 39% of members, and 50% by 63% (Table 2).

Table 2. Numbers and proportions of eligibility criteria of network members in 2010

Criteria number N	Proportion %	Lab number with N	Lab % with N	Lab number with at least N	Lab % with at least N
8	100%	5	13%	5	13%
7	88%	5	13%	10	26%
6	75%	5	13%	15	39%
5	63%	3	8%	18	47%
4	50%	6	16%	24	63%
3	38%	3	8%	27	71%
2	25%	2	5%	29	76%
1	13%	4	11%	33	87%
0	0%	5	13%	38	100%

6. Conclusion

A stable membership of the ICAR Reference Laboratory Network is observed from 2003 but in parallel it is noted the progressive increase of the number of individual members competence. Such increase improve the potential efficiency of the AQA system developed by ICAR from 1996 through more AQA services and expertise proposed to routine testing laboratories in ICAR countries. Nevertheless participation in international proficiency testing schemes organized by ICAR is only the fact of about a half of the network members with a decrease from 2009.
Promotion of the PT programme and technical improvement in the organizing should help to reverse that trend as all the members should be convinced that the most numerous participation in ICAR PTs, the best the quality of performance estimates then the highest the confidence in testing results used to harmonise laboratories and calibration in ICAR member organisations.

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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.

MA SC

ICAR Sub-Commitee on Milk Analysis

ICAR Reference Laboratory Network

- Objectives & Stage of Progress in 2010 -

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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.

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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Major task undertaken by ICAR = Laboratory analytical anchorage

Intent

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

> to harmonise laboratories on a international collective reference

Means

> International guidelines and standards ⇒ harmonised methodology

> Interlaboratory proficiency studies ⇒ lab trueness traceability

> Reference materials ⇒ trueness improvement

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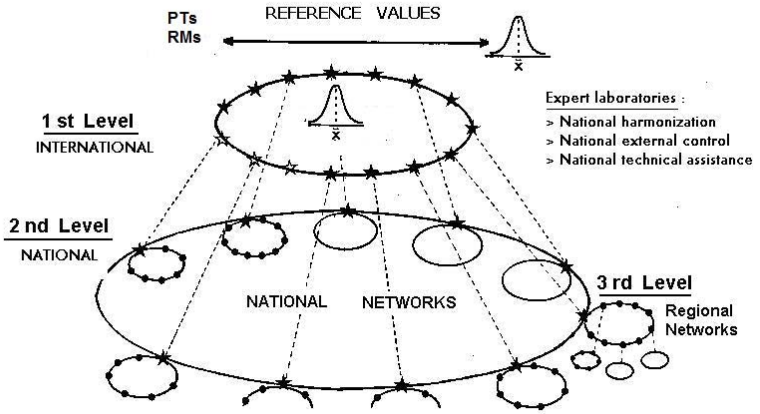
THEORETICAL STRUCTURE

ICAR INTERNATIONAL REFERENCE LABORATORY NETWORK



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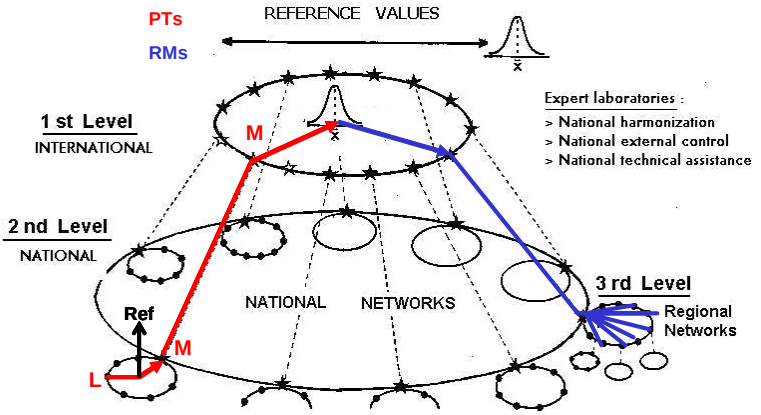
ICAR INTERNATIONAL REFERENCE LABORATORY NETWORK



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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 2010

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

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

(n) : number of member(s)

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

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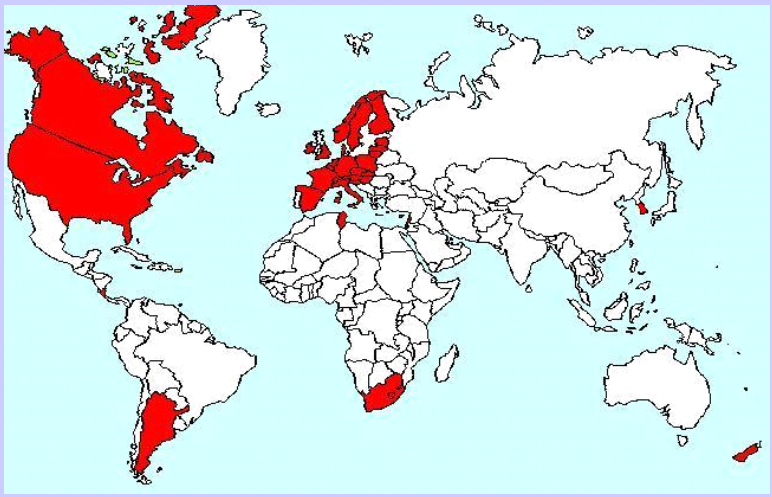
ICAR Reference Laboratory Network

Geographical distribution in 2010



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Evolution of the composition and national roles from 1998 to 2010 (June)

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	24	19	22	24	18	18	13	17	13	3	38

NRTO = National Ring Test Organiser
RMS = Reference Material Supplier
MLCC = Master Laboratory for Centralised Calibratio
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 2010 (June)

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	63	50	58	63	47	47	34	45	34	8	100

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Coverage of eligibility criteria in 2010

Criteria N	Proportion	nr lab with nr	% lab with N	nr lab with at least N	% lab with at least N
8	100%	5	13%	5	13%
7	88%	5	13%	10	26%
6	75%	5	13%	15	39%
5	63%	3	8%	18	47%
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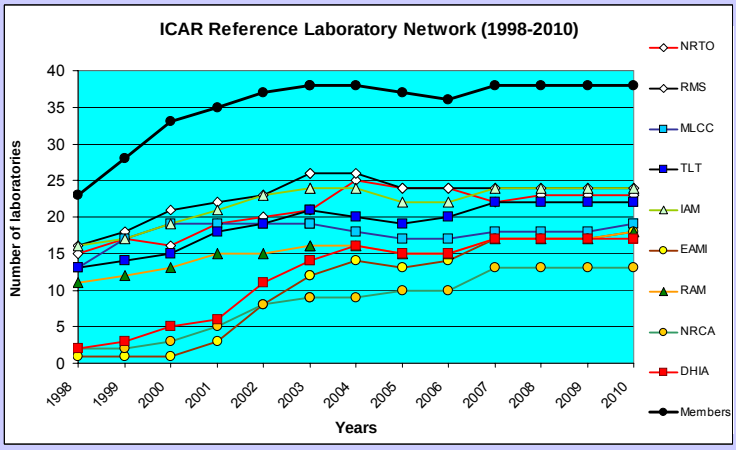
Evolution of membership and missions/activities from 1998 to 2010



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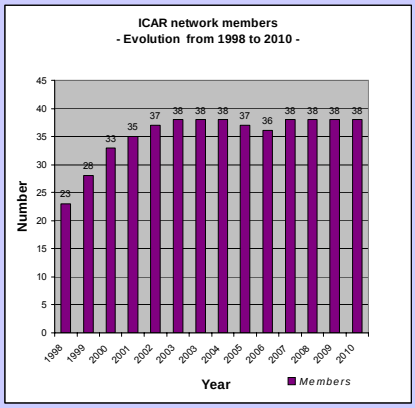
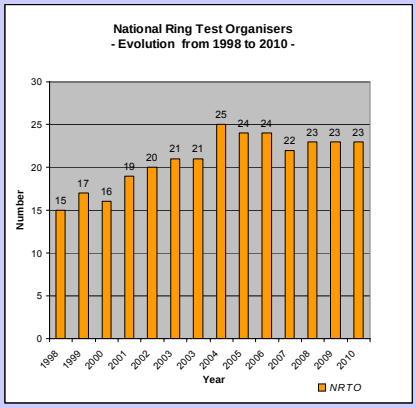
Evolution of membership and missions/activities from 1998 to 2010





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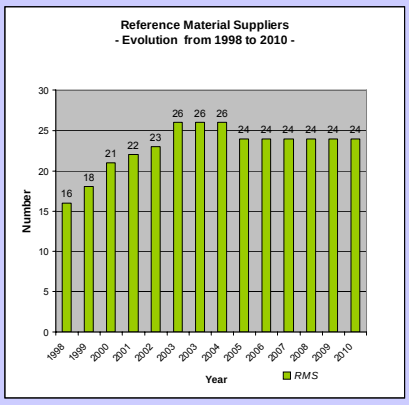

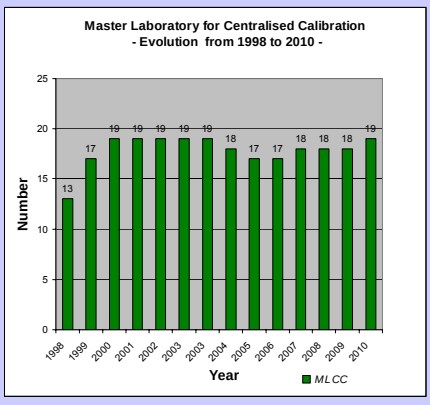

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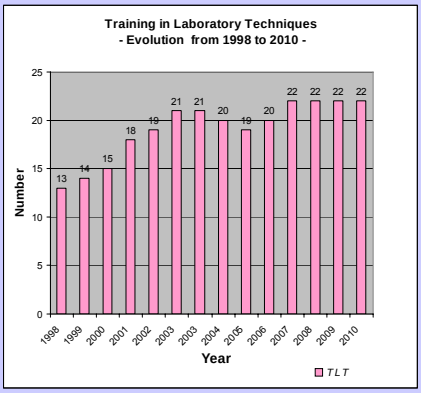

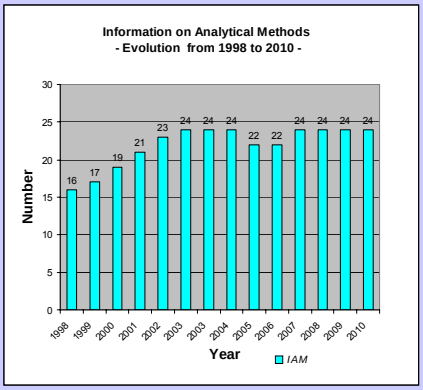

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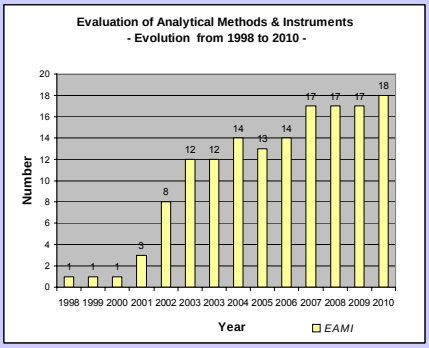
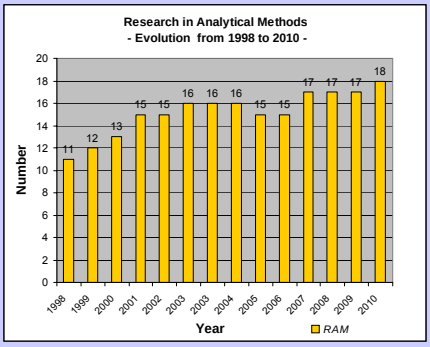
Evolution of membership and missions/activities from 1998 to 2010





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International interlaboratory proficiency studies

From 1996 :	International proficiency scheme organised by ICAR
Frequency :	twice a year
Participants :	15 to 22 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

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From Sousse 2004 and Niagara Falls 2008

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 :** Stability from 2003 around 38 members
  but need to expand more widely for a worldwide recognition purpose with new ICAR members
  e.g. Latin America, Asia and Russia
- **Qualification :** Increase of mission numbers (eligibility criteria)

International Proficiency Testing schemes :

- Regular participation of about 50% of laboratory network members
- participation numbers close to average lab numbers meeting eligibility criteria

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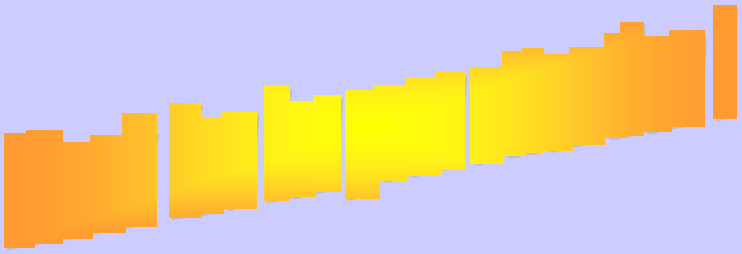

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Analytical precision performance in ICAR proficiency testing programmes

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Abstract

The increased development of international trade in animal genetic has made worldwide comparability and equivalence recognition forf animal performance measurement a topical issue. This is especially the case for milk recording where milk analysis is a major issue.

To assure a harmonised analytical quality among member organisations, ICAR has built up since 1996 a international reference system based on an international network of dairy reference laboratories. Beside a number of recommendation set in guidelines, ICAR organises annually international proficiency testing schemes to help laboratory members of the network to evaluate their analytical performance and, through continuous improving, upgrade the overall analytical precision in the ICAR world and lead to form a consistent group of expert laboratories capable to establish trusty reference values.

Proficiency testing studies are organised twice a year for cow milk and involve reference methods for fat, protein, any methods lactose, urea and somatic cell counting.

A first review of laboratory performances and precision figures for fat, protein and somatic cell counting between 1996 and 2003 were presented in ICAR Session 2004 (Sousse, Tunisia) and showed unsatisfactory precision performances for the group as not conforming to IDF-ISO standard precision values, with inequal individual laboratory performances.

The compared review for the six last year (untill 2009) illustrates a significant improvement with precision figures of the group converging onto or below standard precision values. The number of regular good performing laboratories appears significantly increasing compared to the picture made in 2004.

Nevertheless particular care must maintain and especially be given to somatic cell counting where last trials show a trend to higher precision values.

This work undertaken within ICAR serves as a basis for further undergoing development in the joint IDF-ICAR project “Reference system for somatic cell counting” where qualifying and selecting expert laboratories has become a major issue.

Keywords: milk analysis, dairy laboratories, laboratory network, proficiency

1. Introduction

For the two last decades, the increased development of international trade in animal genetic has made worldwide comparability and equivalence of animal performance measurement a topical issue. This is especially the case for milk recording, being for genetic trade (animals, semen, embryo) or the international evaluation of animal genetic index (Interbull).

To cope with that issue, ICAR set up in 1996 an international network of dairy reference laboratories so as to implement progressively a harmonised international quality assurance system for milk recording analysis worldwide. The reference laboratories are expected to acquire a high expertise in the analytical methods, either standardised or validated, used in milk recording so that they can provide routine laboratories with good reference values through adequate monitoring, anchoring (reference material) and good analytical practices. Harmonising analytical performances within the network is the first major step.

From 1996, ICAR has organised twice a year international proficiency studies for the benefit of the laboratory network members. Those studies were carried out mainly for cow milk and the reference methods for fat, protein, lactose, urea and routine methods for somatic cell counting.

A first review of laboratory performances and the precision figures shown for the methods by the group of laboratories was made in ICAR Session 2004 in Sousse (Tunisia) covering the period from 1996 to 2003 (13 trials). It was the occasion to illustrate how collaborative trials like proficiency studies could be used to strengthen analytical system through measuring analytical performance quality.

Six years later a new review appears necessary to update the knowledge on laboratory performance precision. Indeed, from 2004 and the experience acquired in ICAR, the concept of reference system, including the various uses of laboratory networks, was developed and evolved into the joint IDF-ICAR project "Reference system for somatic cell counting". Qualifying and selecting expert laboratories to provide suitable reference values for somatic cell counting has become a major issue whereas need is still to evaluate the current state of the Art for the main milk components, fat and protein.

2. Material and methods

1.1 Protocol of the PT scheme and organisation

At every end of year the programme of ICAR proficiency testing scheme for the forthcoming year is addressed to the members of the ICAR Reference Laboratory Network and national member organisations of ICAR. The yearly scheme is organised in two rounds, the first in March and the second in September and is applied on cow milk for the component of interest for milk recording and dairy herd management. They are fat and protein by the relevant reference methods, somatic cell counting, lactose and urea by any validated methods excluding infrared. Indeed infrared is marked by significant interferences related to milk composition that makes so-obtained results irrelevant to assess lab performance quality.

Only results for fat, protein and somatic cell counting are reported here as main components used for the genetic evaluation.

1.1.1 Samples

Sets of samples used are made of 10 samples preserved with bronopol at a concentration of 0.02% in milk, covering evenly the range of concentration usually met in routine testing that is
- 10 whole milk samples regularly ranging from 1.5 % to 4.9 % fat
- 10 whole milk samples regularly ranging from 2.5 % to 4.0 % crude protein
- 10 whole milk samples regularly ranging from 4.6 % to 5.1 % lactose
- 10 whole milk samples regularly ranging from 50 to 1600 $x10^3$ cells/ml
- 10 whole milk samples regularly ranging from 10 to 70 mg urea /100 ml

Sample containers are 65 ml or 35 ml polyethylene screw-capped vials with airtight joints to prevent breaking and leakage, and sample temperature before and during shipment to laboratories is +4°C. Possible storage prior analysis is required to be +4°C whereas analysis is to be performed within 5 days for somatic cell counting and 10 days for chemical analysis after the dispatch date.

1.1.2 Milk testing, statistical analysis and assessment parameters

Milk testing is required to be performed in duplicate and according to the current version of the relevant international standard. Cautions for sample preparation before analysis are reminded in an advisory technical note appended to samples. The order of analysis is to be better that one indicated in the numbering in order to avoid errors and reporting is made through adequate tables.

Statistical analysis is performed according to the model developed by the Institut de l'Elevage then used by Cecalait as described in the IDF Bulletin n°342:1999, annexe 3. Assigned values used as reference are calculated according to ISO 13528.

Each sample corresponds to a different concentration level. Assessment is made through dedicated tables allowing the evaluation of lab performance in lines and group performance in columns, for

repeatability (ranges of duplicates and standard deviation of labs or samples, accuracy (means of duplicates, assigned reference per level, differences to assigned reference values, lab scores made of the mean, $\bar{d}$, and the standard deviation of the differences, sd. A synthesis table with lab ranking according to the Euclidian

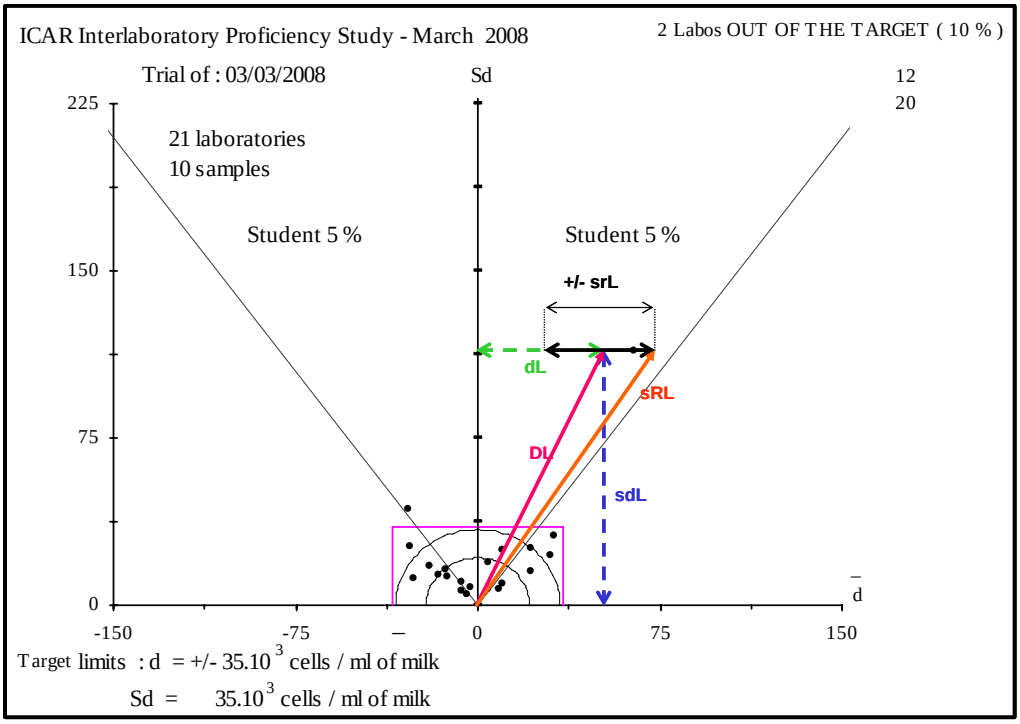

Figure 1. Statistical parameters for individual lab performance evaluation - Example of ICAR trial of March 2008

distance D (as the quadratic mean of the two latter parameters, $D=\sqrt{(\bar{d}^2+sd^2)}$) provides indications of the range of analytical performances and a figures illustrates the related location of each lab vs an indicative conformity target (Figure 1).

For somatic cell counting an table for calibration equation estimates is given as an additional information.

1.2 Meta-analysis for global evaluation

1.2.1 Meta-analysis and group performance evaluation

Meta-analysis consists in gathering individual lab performances in a single large table per criterion - repeatability, mean of bias, standard deviation of difference, distance D – and to present such results in figures in a form of control chart with values in ordinate and trial number in abscissa.

This is done at first with raw data as including all the methods and abnormal scores for a first visual scrutiny and evaluation of the evolution of performance throughout time, then after discarding not expected methods (e.g. Gerber, infrared methods) and outlier laboratories.

If the source of outliers are evident and reflects only a basic error of unit or disorder than can be repaired, correction and recalculation of scores are made since they would be detected in lab situation when calibrating routine analysers. Otherwise outlier are detected then discarded through a Cochran test applied on the distance D with a risk of error of 1% and in the limit of 20% max which was attained or passed but occasionally.

As well the quadratic averaging per trials of all the participant values serve to evaluate the precision figures for each trial and the similar integration of the individual trial precision figures for a defined period of time allow to measure the overall precision improvement of the group of laboratories (Table 1).

Table 1. Robust estimates of precision and accuracy parameters in fat, protein and somatic cell counting by meta-analysis in ICAR trials

Fat (g/kg)

Period	1996 - 2009	1996 - 2003	2004 - 2009	2008 - 2009
Mean Sr	**0,09**	0,09	0,08	0,07
Mean d	**-0,01**	-0,01	0,00	0,00
Mean Sd	**0,16**	0,18	0,12	0,10
Mean D	**0,20**	0,23	0,16	0,13
Mean SR	**0,21**	0,24	0,17	0,14
Number N	**387**	203	184	60

Protein (g/kg)

Period	1996 - 2009	1996 - 2003	2004 - 2009	2008 - 2009
Mean Sr	**0,11**	0,12	0,10	0,10
Mean d	**-0,05**	-0,07	-0,02	-0,02
Mean Sd	**0,14**	0,16	0,11	0,11
Mean D	**0,23**	0,27	0,17	0,17
Mean SR	**0,25**	0,29	0,19	0,18
Number N	**428**	239	189	60

Somatic cell counting (1000 cells/ml)

Period	1996 - 2009	1996 - 2003	2004 - 2009	2008 - 2009
Mean Sr	**18,8**	17,4	13,7	14,4
Mean d	**-1,6**	+2,7	-0,4	-12,1
Mean Sd	**42,1**	44,1	24,7	30,9
Mean D	**54,7**	55,7	34,1	43,4
Mean SR	**57,2**	57,9	36,2	45,2
Number N	**254**	111	143	50

1.2.2 Meta-analysis and lap performance evaluation

Individual lab control chart can be built up to assess each lab over a period of time and the calculation of an average score for the defined period (for instance the four last trials) becomes possible for a defined period of time to apply a suitable selection of laboratories for a defined purpose according to their performances (Tables 2, 3, 4). Lab participation frequency can be utilized as an additional selection criterion since more participation for a same average value ascertains better performance regularity.
Besides, similarly as shown in Sousse (2004), such individual score merging allow to calculate a robust true individual uncertainty for the measurement applied for a given representative period.

3. Results

3.1 Overall scrutiny of individual scores

Compared to the period of 1996 to 2003, the control charts have shown in general a lower frequency of outliers with lower upper values for the period of 2004 to 2009, can this be for repeatability standard deviation, mean bias, standard deviation of bias or Euclidian distance D.

The outlier discarding suppressed less scores and rarely reached the percentage limit for deletion of 20%.

3.2 Repeatability and reproducibility figures (precision)

3.2.1 Fat measurement

Repeatability standard deviation values, sr, were higher than 0.10 g/kg from 1996 to 1999 then decreased to keep almost stable from 2000 to 2006 just above the standard limit of 0.07 g/kg of IDF 1 / ISO 1211. From 2007, in conjunction with the implementation of ICAR Quality Certificate, the values dropped below the limit. Referring to new standard value 0.15 g/kg implemented with the recent revision of IDF 1 / ISO 1211 the group shows good compliance from 1996.

A similar trend is observed for reproducibility standard deviation, sR, although the group never passed through the standard reproducibility limit of 0.14 g/kg. Referring to new standard value 0.20 g/kg implemented with the recent revision of IDF 1 / ISO 1211 the group shows however frequent compliance from 2000 (Figure 2).

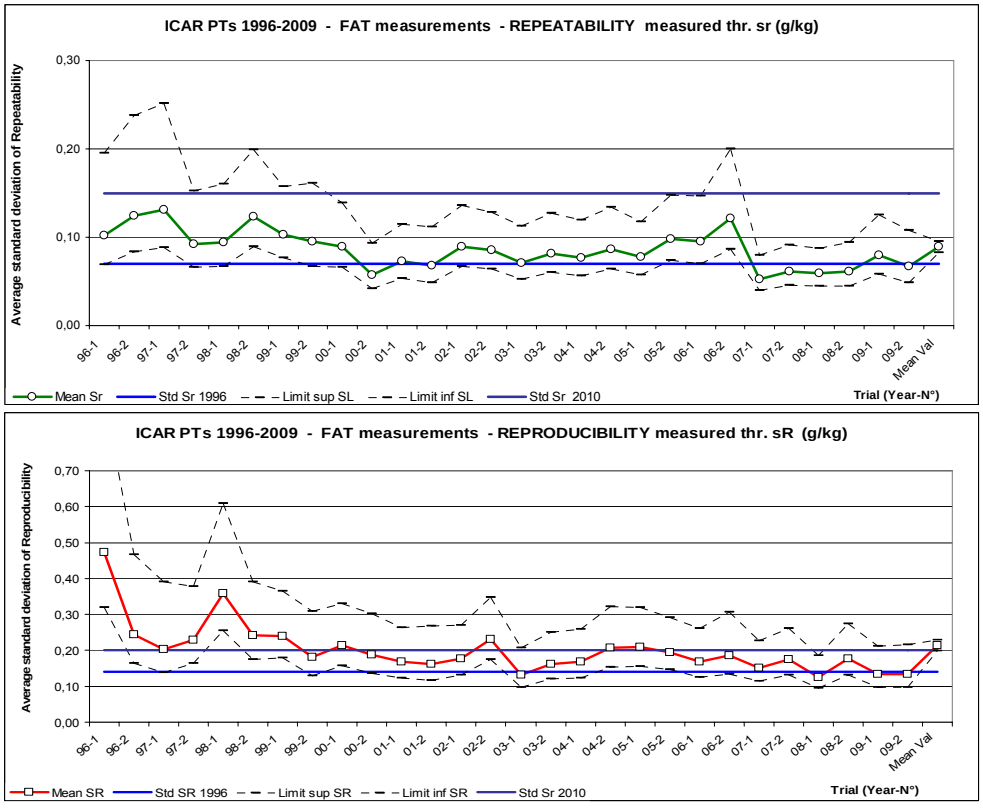

Figure 2. Precision figures of fat analysis in ICAR trials between 1996 and 2009

3.2.2 Protein measurement

From 1996 to 1999 repeatability standard deviation values are mostly lower than the standard limit of 0.14 g/kg of IDF 20 / ISO 8968 with only two trials outside. However stabilization around 0.10 g/kg from 2006 is observed.

Reproducibility standard deviation was significantly higher than the standard limit of 0.18 g/kg of IDF 20 / ISO 8968 but from 2003 have decreased to a regular fitting onto the limit (Figure 3).

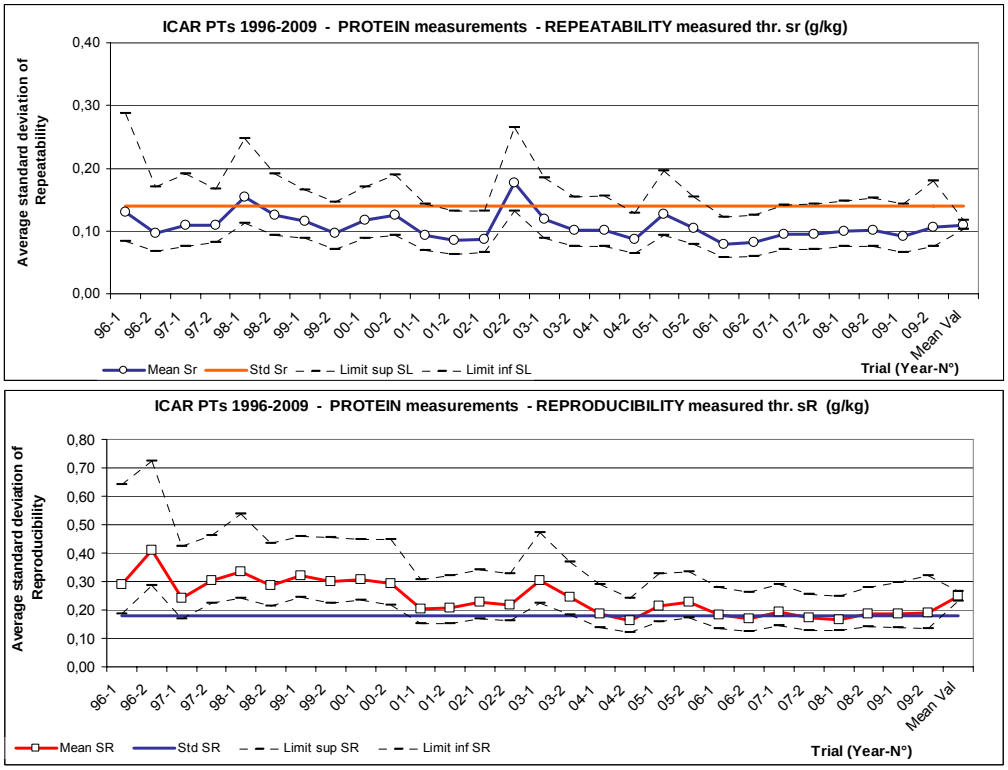

Figure 3. Precision figures of protein analysis in ICAR trials between 1996 and 2009

3.2.3 Somatic cell counting

From 1996 to 1999 repeatability standard deviation values are mostly significantly lower than the standard limit of 20 000 cells/ml of IDF 148-2 / ISO 13366-2 with only one trial outside in 2000. However after a optimal performance period between 2005 and 2007 irregular discrepancy is observed.

Besides, whereas reproducibility standard deviation was at the level or higher than the standard limit of 45 000 cells/ml of IDF 148-2 / ISO 13366-2 until 2003, they have been reduced significantly around a level of 30 000 cells/ml from 2004 to 2008. Since a deterioration of the global performance is observed and the limit is passed through in 2009 (Figure 4).

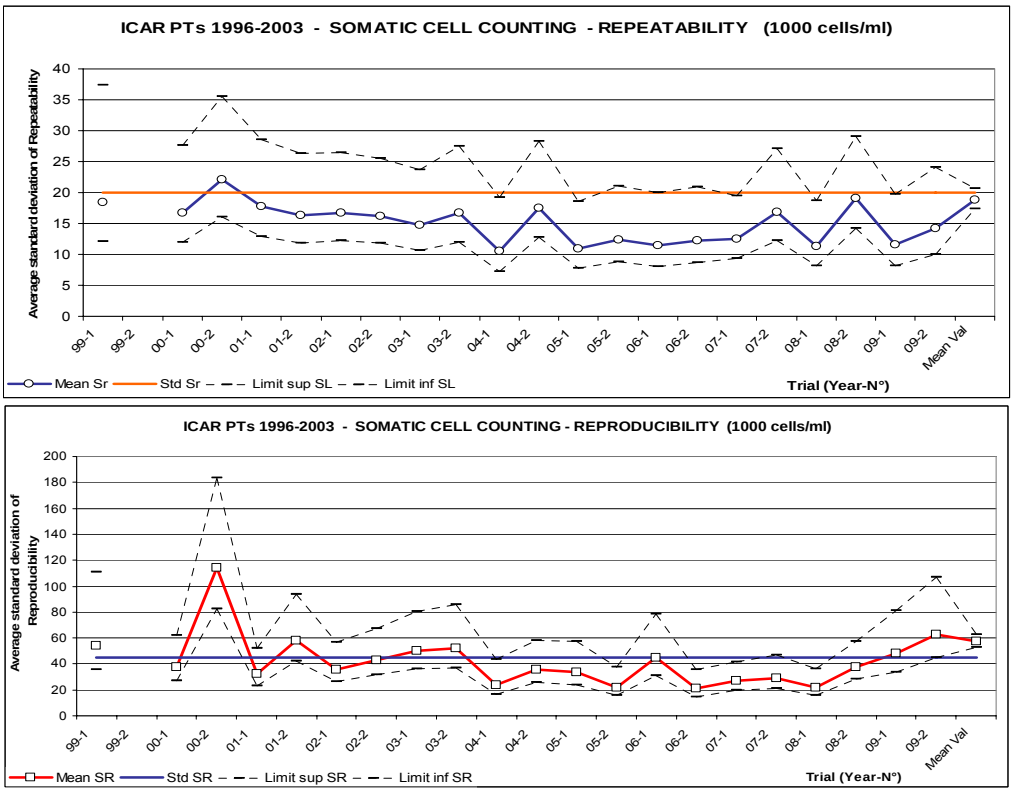

Figure 4. Precision figures of somatic cell counting in ICAR trials between 1996 and 2009

3.3 Individual lab performances and reference lab selection

Individual performances whatever the component - fat, protein or somatic cells – have shown significant progress as illustrate through the improvement of the overall precision of the group of participants.

Selection of group of reference lab for a defined purpose such as assigning reference values for reference material should be made from the more recent lab performances measured hence to define the last necessary period. Then to rank laboratories according to the overall precision shown by a significant statistical parameter, for instance sRL which covers all the sources of errors of the laboratory.

Tables 2. 3 and 4 show examples respectively for fat, protein and somatic cell analysis for the four last trials (2008-2009) where yellow highlighting indicates prior defined limits are passed. On such a basis, one could assumed to retain 19 labs of 21 for fat, 17 of 20 for protein and 12 of 18 in somatic cell counting.

Nevertheless not all participated at the same time in the same trials and the observed participation frequency (PT %) indicates regularity of the feedback information and possible corrective actions. So ranking according to the frequency can be associated to the ranking on scores so as to introduced a weighting in the laboratory selection.

Table 2. Laboratory ranking according to four trial average reproducibility performances (sR) in ICAR trials – Fat results

RANK	PT %	Mean sr	Mean $\bar{d}$	S mean $\bar{d}$	Mean sd	Mean D	Mean sR
1	100%	0,04	0,02	0,09	0,05	0,06	0,07
2	75%	0,04	0,05	0,06	0,04	0,06	0,07
3	75%	0,04	0,02	0,08	0,06	0,07	0,07
4	100%	0,04	0,04	0,07	0,05	0,07	0,07
5	50%	0,07	0,01	0,12	0,06	0,07	0,08
6	50%	0,04	0,04	0,05	0,07	0,10	0,10
7	100%	0,07	-0,03	0,06	0,07	0,09	0,10
8	100%	0,07	-0,01	0,06	0,06	0,09	0,10
9	100%	0,05	0,07	0,31	0,08	0,12	0,12
10	50%	0,04	0,11	0,05	0,08	0,14	0,14
11	100%	0,07	-0,11	0,18	0,08	0,15	0,15
12	100%	0,06	-0,05	0,61	0,14	0,16	0,17
13	100%	0,06	0,01	0,18	0,09	0,17	0,18
14	100%	0,12	0,01	0,19	0,16	0,17	0,19
15	25%	0,16	0,18	0,14	0,06	0,19	0,22
16	50%	0,07	-0,09	0,08	0,21	0,23	0,23
17	100%	0,07	-0,16	0,10	0,16	0,24	0,24
18	75%	0,09	0,01	0,18	0,28	0,35	0,36
19	50%	0,11	0,04	0,21	0,35	0,36	0,37
20	100%	0,05	-0,13	0,24	0,12	0,38	0,38
21	25%	2,28	-1,16	0,78	1,81	2,15	2,69

Table 3. Laboratory ranking according to four trial average reproducibility performances (sR) in ICAR trials – Protein results

RANK	PT %	Mean sr	Mean $\bar{d}$	S mean $\bar{d}$	Mean sd	Mean D	Mean sR
1	100%	0,05	-0,02	0,11	0,08	0,09	0,10
2	50%	0,07	-0,01	0,11	0,07	0,10	0,11
3	25%	0,16	0,08	0,12	0,12	0,00	0,11
4	25%	0,03	-0,02	0,07	0,11	0,12	0,12
5	100%	0,09	0,09	0,09	0,07	0,12	0,13
6	100%	0,06	0,01	0,14	0,08	0,13	0,14
7	75%	0,09	-0,09	0,19	0,08	0,13	0,14
8	100%	0,06	0,04	0,08	0,09	0,14	0,15
9	75%	0,12	0,05	0,14	0,09	0,13	0,16
10	100%	0,05	-0,04	0,20	0,08	0,16	0,16
11	100%	0,16	-0,01	0,09	0,11	0,15	0,18
12	100%	0,10	-0,10	0,36	0,12	0,19	0,21
13	50%	0,10	0,04	0,17	0,22	0,22	0,24
14	75%	0,10	0,16	0,15	0,17	0,23	0,24
15	100%	0,09	-0,12	0,12	0,12	0,23	0,24
16	75%	0,17	-0,20	0,52	0,09	0,25	0,27
17	100%	0,08	-0,28	0,10	0,07	0,31	0,32
18	100%	0,21	-0,20	0,31	0,24	0,39	0,41
19	100%	0,12	-0,30	0,41	0,74	0,87	0,88
20	100%	0,10	-0,39	0,33	1,61	1,73	1,73

Table 4. Laboratory ranking according to four trial average reproducibility performances (sR) in ICAR trials – Somatic cell counting results

RANK	PT %	Mean sr	Mean $\bar{d}$	S mean $\bar{d}$	Mean sd	Mean D	Mean sR
1	100%	13,4	5,8	12,5	15,5	19,3	21,5
2	25%	12,0	11,0	3,5	17,0	20,2	22,0
3	100%	6,3	-0,3	24,2	14,5	22,6	23,0
4	100%	8,0	-11,3	19,6	14,8	22,3	23,0
5	100%	10,6	-11,5	42,9	18,5	22,8	24,0
6	100%	14,3	10,5	19,8	22,7	29,4	31,1
7	100%	7,0	-2,5	24,2	30,6	34,2	34,5
8	50%	9,6	-22,5	26,6	24,5	36,7	37,4
9	100%	24,6	3,3	35,7	26,8	37,0	40,8
10	75%	19,4	-31,0	100,9	26,8	44,2	46,3
11	75%	11,9	-32,3	17,8	31,5	47,4	48,2
12	50%	10,6	-22,0	20,2	49,4	54,4	54,9
13	25%	7,0	-43,0	41,0	41,0	59,4	59,6
14	100%	13,3	-44,8	36,9	52,6	77,9	78,4
15	100%	22,9	-51,5	27,7	56,5	81,9	83,5
16	100%	16,4	54,5	41,1	57,8	83,6	84,4
17	50%	15,6	-57,5	36,7	76,5	98,1	98,7
18	75%	69,3	-7,0	51,5	85,6	102,8	113,9

4. Conclusion

Findings of the first review and their presentation in Sousse 2004 permitted to inform laboratory network for the need to improve testing practices and invited them to review own ways of work. This has resulted in a effective improvement at individual lab levels and consequently at the level of the whole group of participants. The efficiency of the ICAR reference system is demonstrated there. Year 2006 saw the implementation of the ICAR Quality Certificate to replace the Special Stamps and broaden the scope of ICAR quality assurance system to all the parts of its expertise and among them milk analysis. Correlatively tighter regularity and compliance took place for fat and protein, at a lower extent for somatic cell count, demonstrating the efficiency of ICAR quality policy.

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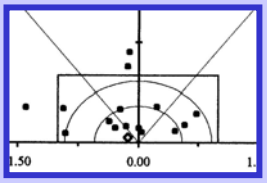

International proficiency studies in ICAR Reference Laboratory Network

Review from 1996 to 2009

Olivier Leray, CECALAIT, France

SUMMARY

- General
- Protocol of proficiency studies
- Data treatment in the review
- Participation
- Review of performances : Fat, protein and SCC
- Alternative use of PTs results:

ICAR International Interlaboratory Proficiency Studies

GENERAL

- **From 1996 to 2009 :**
  - 28 trials for Fat, Protein & Lactose
  - 21 trials for Somatic Cell Counting
  - 20 trials for Urea
- **Dealt with in the review :** Fat, Protein & Somatic Cell Counting

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Proficiency study protocol

- Samples : 10 cow milk samples dispatched to participants by international express carriers, analysed in duplicate by laboratories using :
  - reference methods used to calibrate routine methods (infra red spectrophotometers) for fat and protein,
  - routine methods for somatic cell counting.
- Milk component levels : concentration ranges for instrument calibration (1.5-4.9 % F, 2.5-4.0 % P, 50-1600 .1000 cells/mL).
- Standard statistical treatment : (IDF Bulletin n°342:1999, annexe 3).
  - by the organiser
  - to extract and provide information about **precision** and **trueness** of **participants** and the **group** of laboratories

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Element of assessment
- Statistical parameters -

- Precision of the laboratory :
  - standard deviation of repeatability of the laboratory SL (= SrL) calculated from duplicates (ISO 8196 | IDF 128)
  - Euclidian distance D equivalent to overall prediction error when duplicates ($D = \sqrt{\bar{d}^2 + Sd^2}$),
  - Standard deviation of reproducibility SRL of the laboratory ($SRL = \sqrt{\overline{SrL}^2/2 + \bar{d}^2 + Sd^2}$),
- Trueness of participants :
  - mean of differences $\bar{d}$ between means of duplicates duplicate and assigned reference values
  - standard deviation of differences Sd.
- Outlier discarding for the overall precision assessment :
  - Cochran test applied to D values

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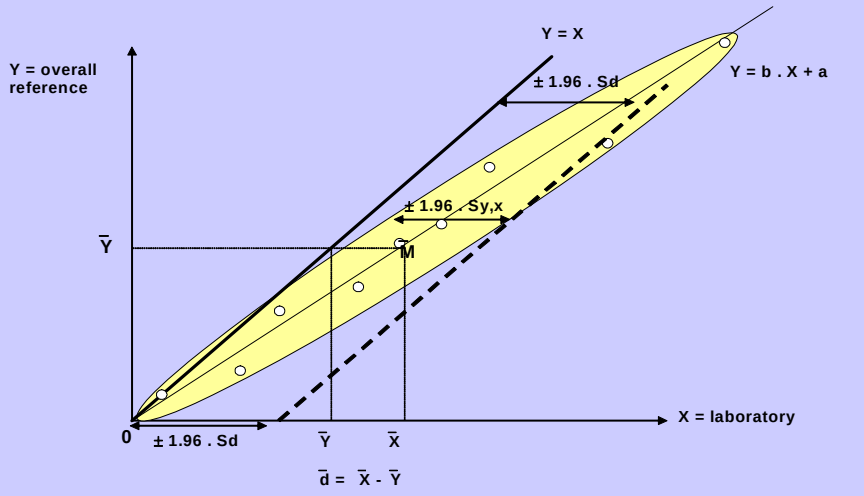
Statistical parameters to evaluate lab trueness performances and calibration



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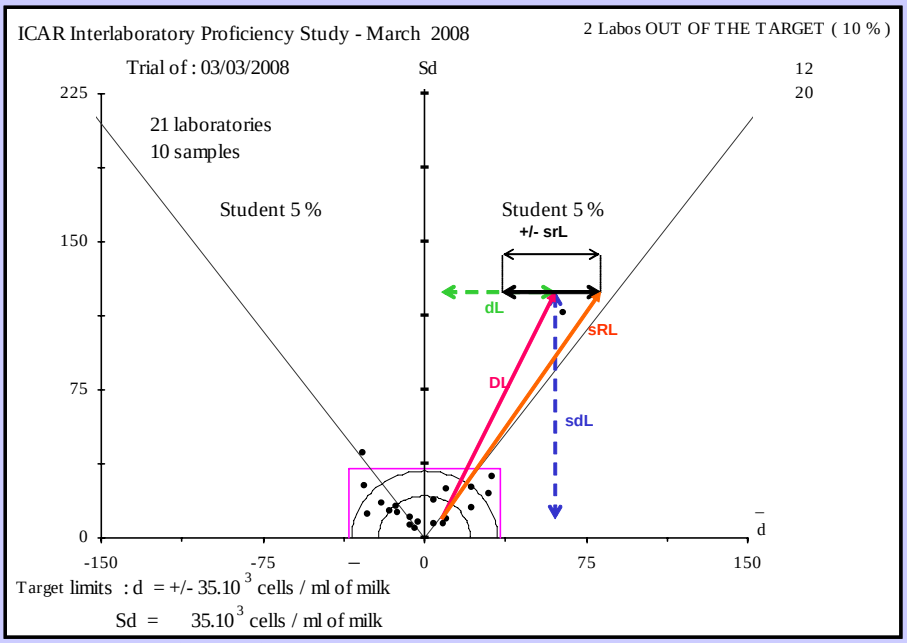

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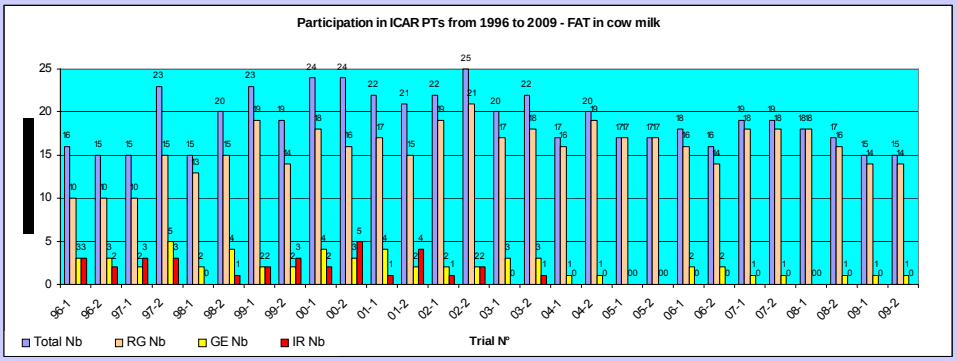
Participation in PTs for FAT from 1996 to 2009



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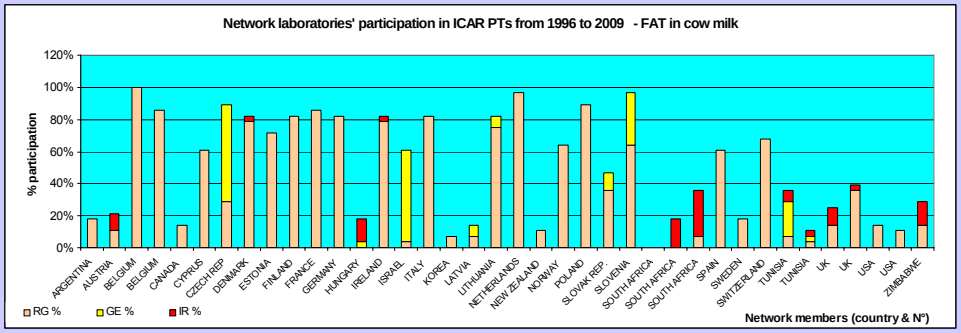
Participation in PTs for FAT from 1996 to 2009



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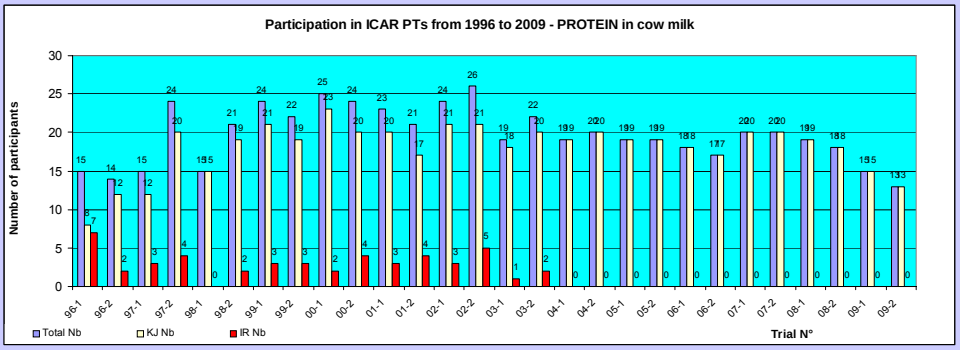
Participation in PTs for PROTEIN from 1996 to 2009



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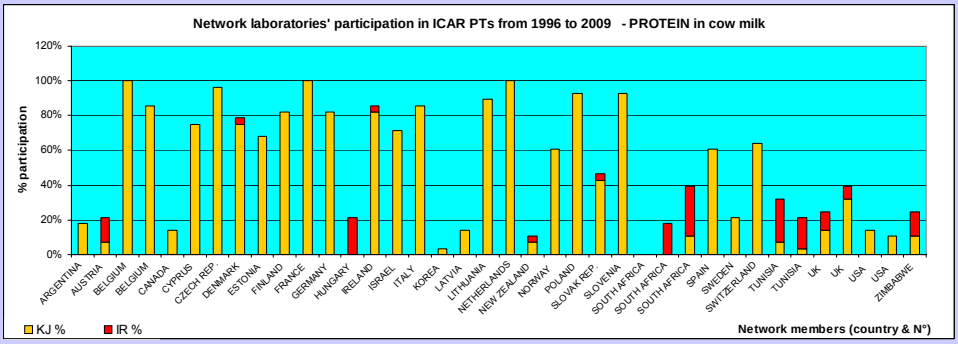
Participation in PTs for PROTEIN from 1996 to 2009



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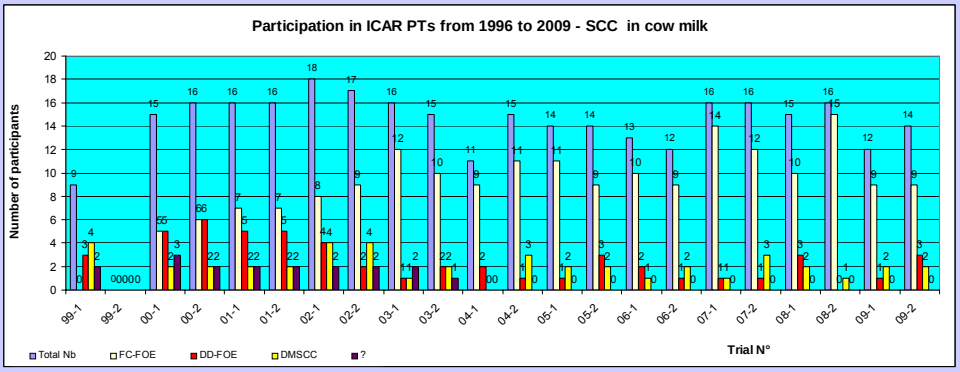
Participation in PTs for SOMATIC CELL COUNTING from 1996 to 2009



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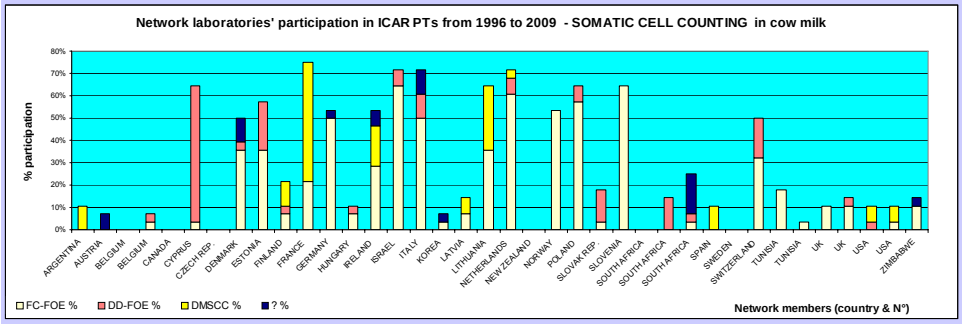
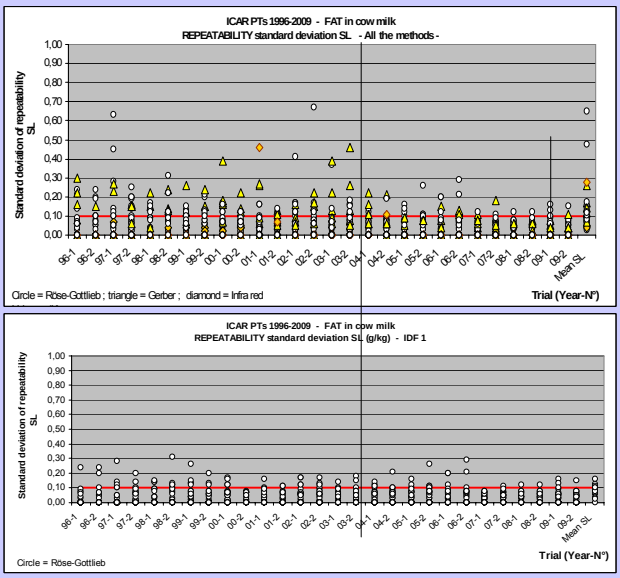
Participation in PTs for SOMATIC CELL COUNTING from 1996 to 2009



Overview of statistical parameter results with comparing :

- all the methods, all the data
- after discarding routine methods and « outliers »

Repeability of FAT measurements



Sr = 0.07 g/Kg limit = 0.10 g/Kg

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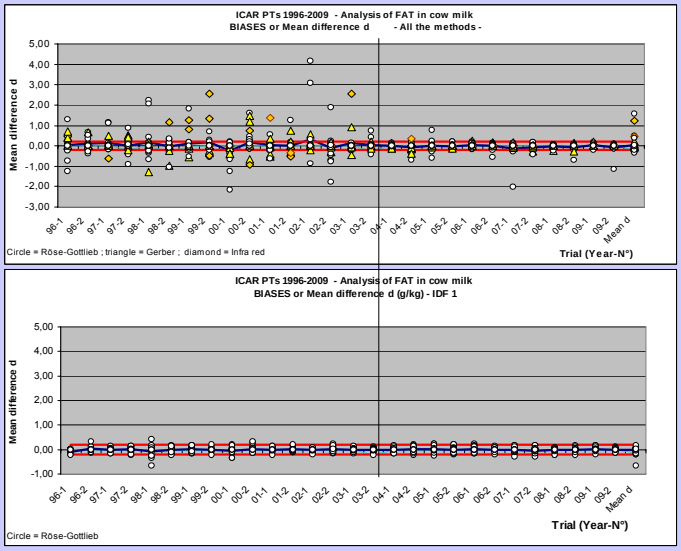
Mean differences $\bar{d}$ for FAT



Standard SR = 0.14 g/Kg Tolerance limit = +/- 0.20 g/Kg

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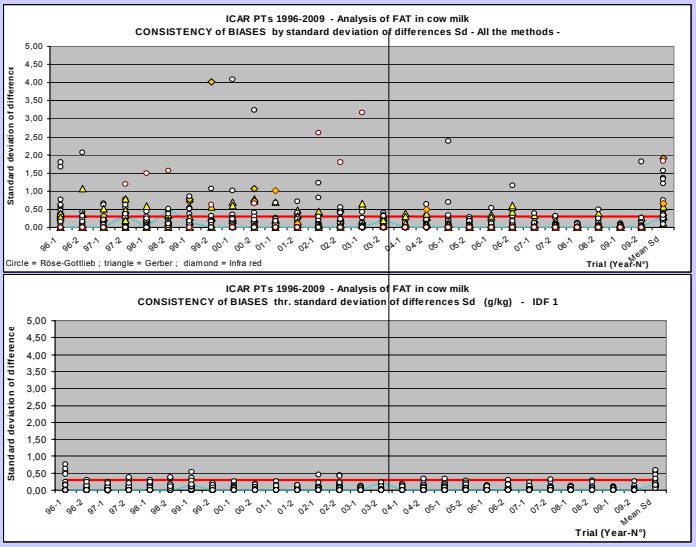
Standard deviation of differences Sd for FAT



Tolerance limit = 0.30 g/Kg

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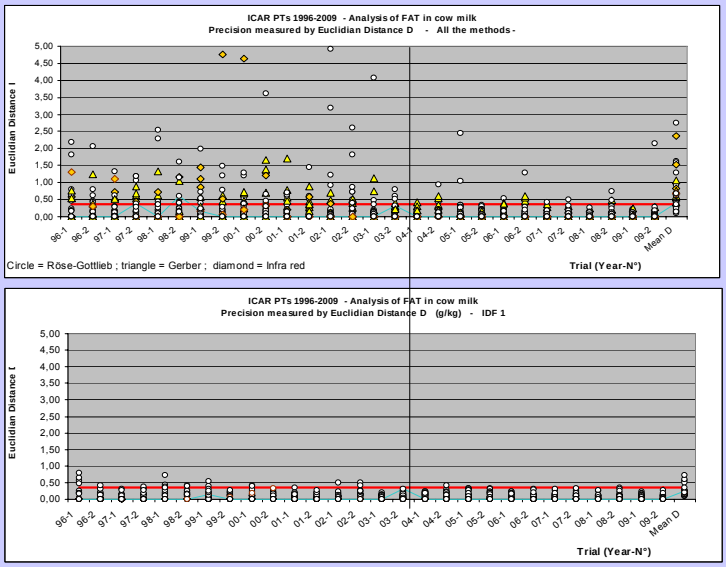
Euclidian distance D (Prediction Error) for FAT



Tolerance limit = 0.36 g/Kg

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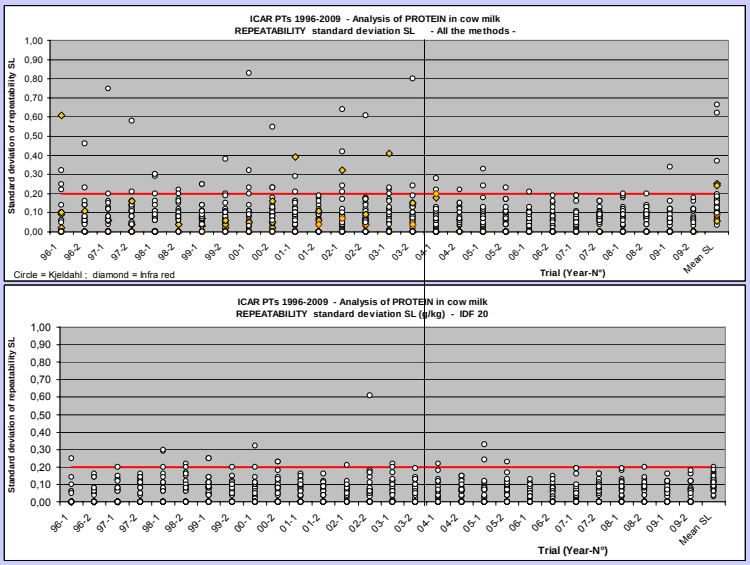
Repeability of PROTEIN measurements



Standard Sr = 0.14 g/Kg Tolerance limit = 0.20 g/Kg

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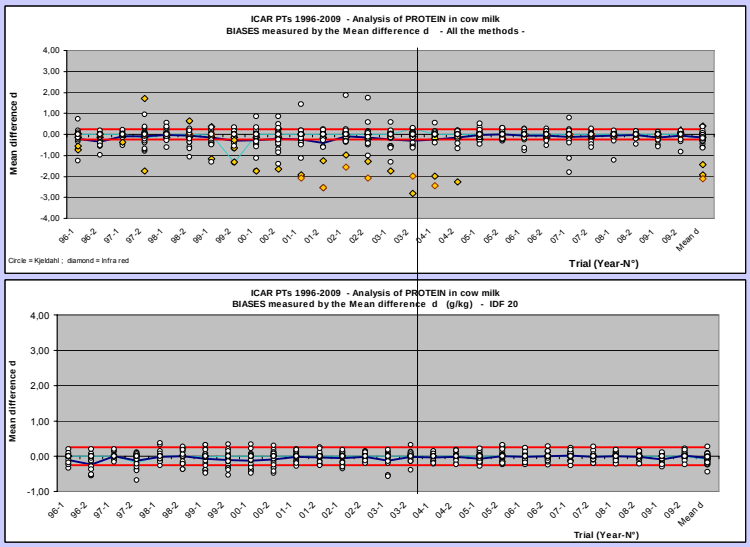
Mean differences $\bar{d}$ for PROTEIN



Standard SR = 0.18 g/Kg Tolerance limit = +/- 0.25 g/Kg

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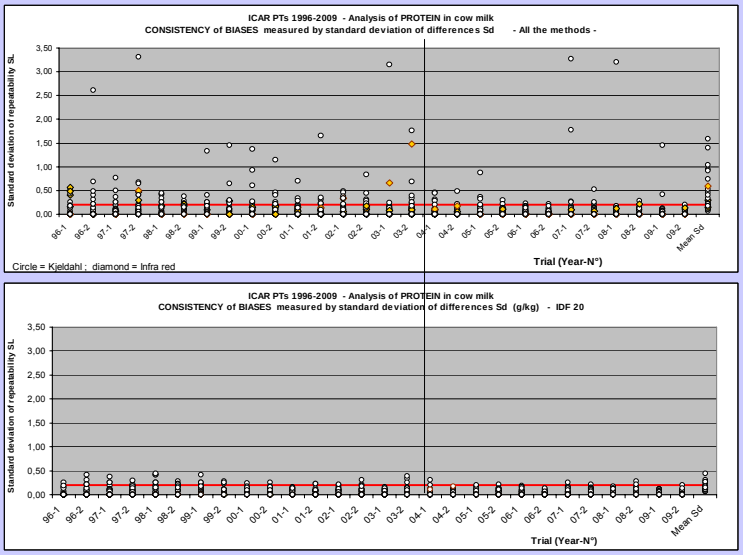
Standard deviation of differences Sd for PROTEIN



Tolerance limit = 0.20 g/Kg

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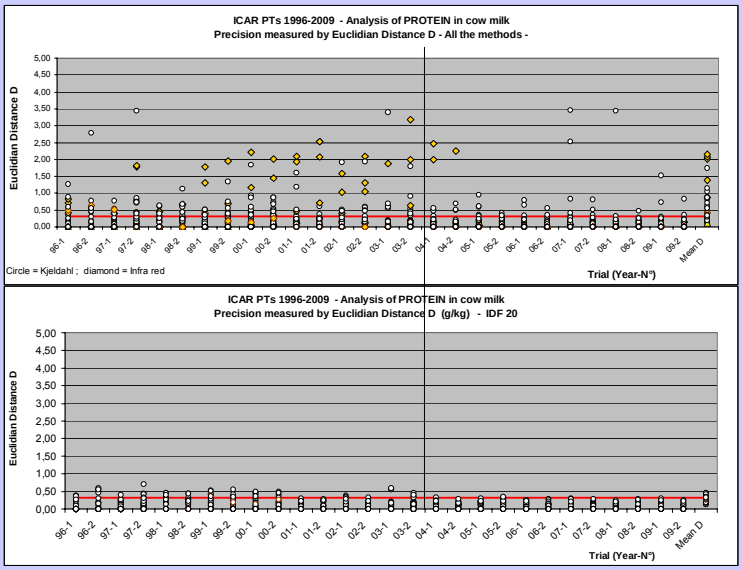
Euclidian distance D (Prediction Error) for PROTEIN



Tolerance limit = 0.32 g/Kg

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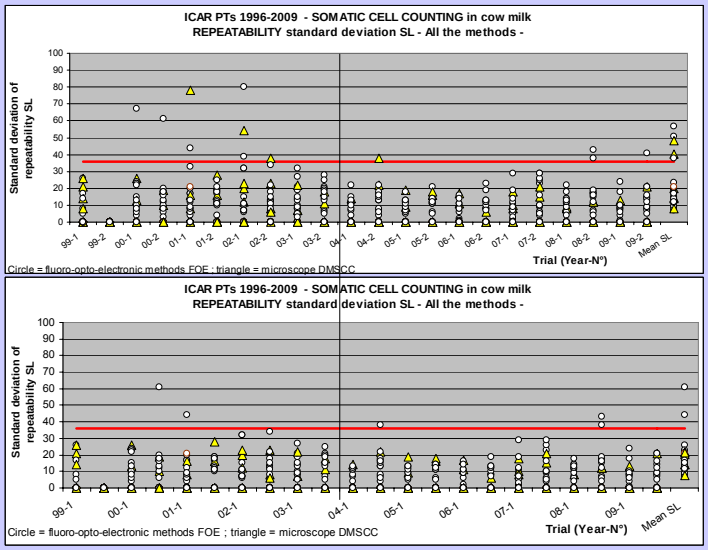
Repeability of SCC measurements



Standard Sr = 20 000 cells/mL Tolerance limit = 36 000 cells/mL

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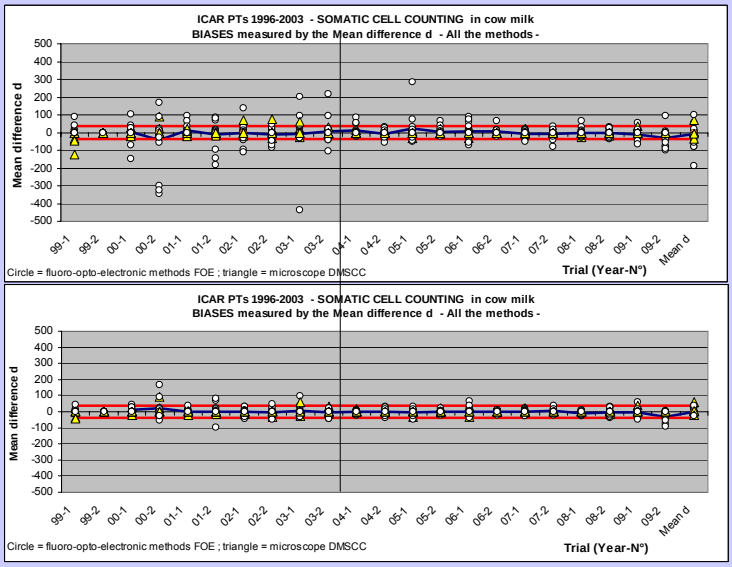
Mean differences $\bar{d}$ for SCC



Standard SR = 50 000 cells/mL Tolerance limit = +/- 35 000 cells/mL

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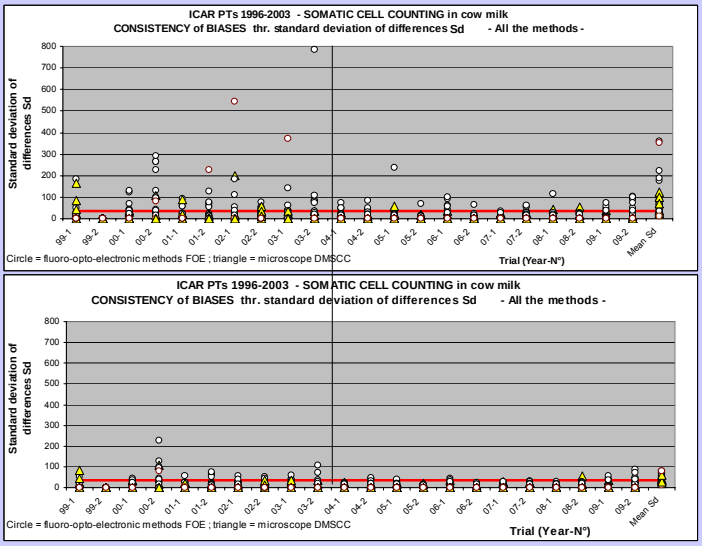
Standard deviation of differences Sd for SCC



Tolerance limit = 35 000 cells/mL

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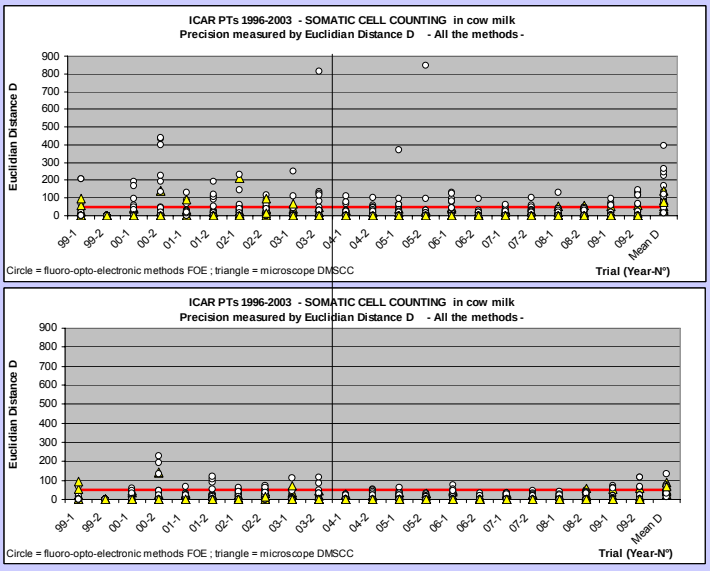
Euclidian distance D (Prediction Error) for SCC



Tolerance limit = 50 000 cells/mL

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Precision characteristics of methods within the network

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Meta-analysis on 1996-2009

ICARPTs 1996-2009: Overall precision of FAT measurements - All the methods and results

NYCR	951	952	9F1	9F2	981	982	991	992	001	002	011	012	021	022	031	032	041	042	051	052	061	062	0F1	0F2	081	082	091	092	MeanVal
MeanSL	0.10	0.12	0.13	0.09	0.09	0.12	0.10	0.10	0.09	0.06	0.07	0.07	0.09	0.09	0.07	0.08	0.08	0.09	0.08	0.10	0.09	0.12	0.05	0.06	0.06	0.06	0.08	0.07	0.09
Mean d	-0.09	0.05	-0.01	0.01	-0.07	-0.01	0.02	-0.01	-0.03	0.02	0.00	0.01	-0.02	0.01	0.00	-0.02	-0.02	0.01	0.01	-0.02	0.01	0.00	-0.02	-0.03	-0.01	-0.01	0.03	-0.01	-0.01
MeanSd	0.45	0.16	0.14	0.18	0.18	0.18	0.20	0.13	0.12	0.10	0.12	0.10	0.15	0.19	0.09	0.12	0.11	0.14	0.14	0.14	0.10	0.14	0.09	0.12	0.07	0.14	0.07	0.10	0.16
MeanD	0.47	0.22	0.18	0.22	0.24	0.22	0.23	0.17	0.20	0.18	0.16	0.15	0.16	0.22	0.12	0.15	0.16	0.19	0.20	0.18	0.15	0.16	0.14	0.17	0.12	0.17	0.12	0.12	0.20
MeanSR	0.47	0.24	0.20	0.25	0.26	0.24	0.24	0.18	0.21	0.19	0.17	0.16	0.18	0.23	0.13	0.16	0.17	0.21	0.21	0.20	0.17	0.19	0.15	0.17	0.13	0.18	0.13	0.13	0.21
NinterN	8	8	8	12	11	13	16	11	15	13	14	12	16	17	14	15	15	15	16	17	15	12	17	17	18	15	14	13	233

ICARPTs 1996-2009: Overall precision of PROBEN measurements - All the methods and results

NYCR	951	952	9F1	9F2	981	982	991	992	001	002	011	012	021	022	031	032	041	042	051	052	061	062	0F1	0F2	081	082	091	092	MeanVal
MeanSL	0.13	0.10	0.11	0.11	0.15	0.13	0.12	0.10	0.12	0.12	0.09	0.08	0.09	0.18	0.12	0.10	0.10	0.09	0.13	0.10	0.08	0.08	0.09	0.10	0.10	0.10	0.09	0.11	0.11
Mean d	-0.10	-0.23	-0.01	-0.13	-0.02	-0.01	-0.08	-0.11	-0.13	-0.09	-0.03	-0.03	-0.06	-0.02	-0.12	-0.01	-0.03	-0.02	-0.06	0.01	-0.01	0.00	0.01	-0.02	0.00	-0.02	-0.09	0.02	-0.05
MeanSd	0.16	0.20	0.19	0.14	0.25	0.17	0.17	0.14	0.12	0.13	0.11	0.12	0.12	0.13	0.10	0.17	0.12	0.10	0.11	0.10	0.10	0.07	0.12	0.11	0.11	0.14	0.09	0.11	0.14
MeanD	0.26	0.40	0.23	0.29	0.31	0.27	0.30	0.29	0.29	0.27	0.19	0.19	0.21	0.18	0.29	0.23	0.17	0.15	0.19	0.21	0.17	0.15	0.17	0.16	0.15	0.17	0.17	0.17	0.23
MeanSR	0.29	0.41	0.24	0.30	0.33	0.29	0.32	0.30	0.31	0.29	0.20	0.21	0.23	0.22	0.30	0.24	0.19	0.16	0.21	0.23	0.18	0.17	0.19	0.17	0.17	0.19	0.19	0.19	0.25
NinterN	6	10	10	16	13	16	21	16	19	16	16	15	17	17	15	16	15	17	15	18	15	15	17	17	18	17	14	11	239

ICARPTs 1996-2009: Overall precision of SOMATIC CELL COUNTING - All the methods

NYCR	951	952	9F1	9F2	981	982	991	992	001	002	011	012	021	022	031	032	041	042	051	052	061	062	0F1	0F2	081	082	091	092	MeanVal
MeanSL							18		17	22	18	16	17	16	15	17	11	18	11	12	11	12	13	17	11	19	12	14	19
Mean d							-2		10	21	0	2	0	-8	8	-6	-2	1	-3	-1	3	2	-2	4	-8	-3	-7	-30	-2
MeanSd							43		29	92	24	38	26	30	29	43	18	28	22	14	26	15	19	21	14	27	32	44	42
MeanD							52		35	112	29	55	33	41	48	50	22	34	32	20	43	19	24	25	20	35	46	61	55
MeanSR							54		38	114	32	58	36	43	50	52	24	36	34	22	45	21	27	29	22	38	48	63	57
NinterN							7		12	13	13	13	14	14	13	12	9	12	11	11	10	11	15	13	12	16	11	11	111

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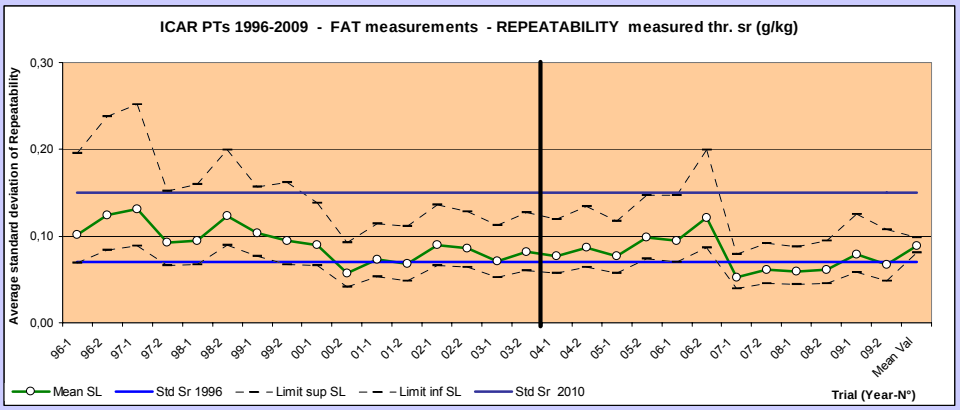
Precision for FAT



Standard sr 1996 = 0.07 g/Kg

Standard sr 2010 = 0.15 g/Kg

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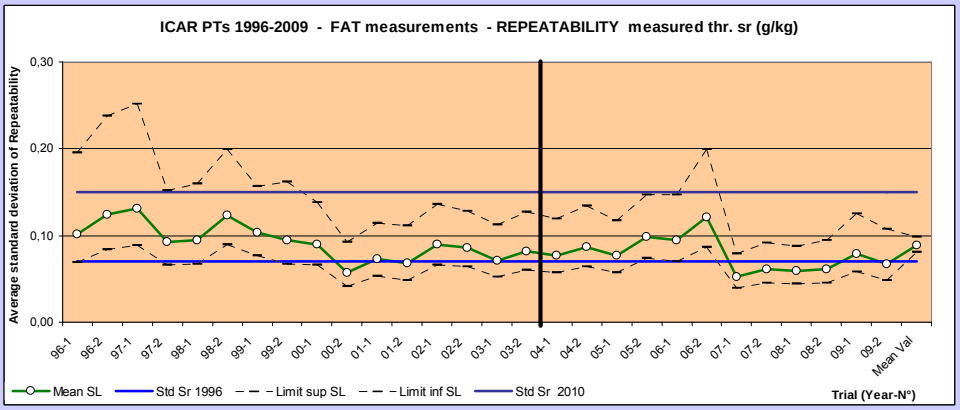
Precision for FAT



Standard sr 1996 = 0.07 g/Kg

Standard sr 2010 = 0.15 g/Kg

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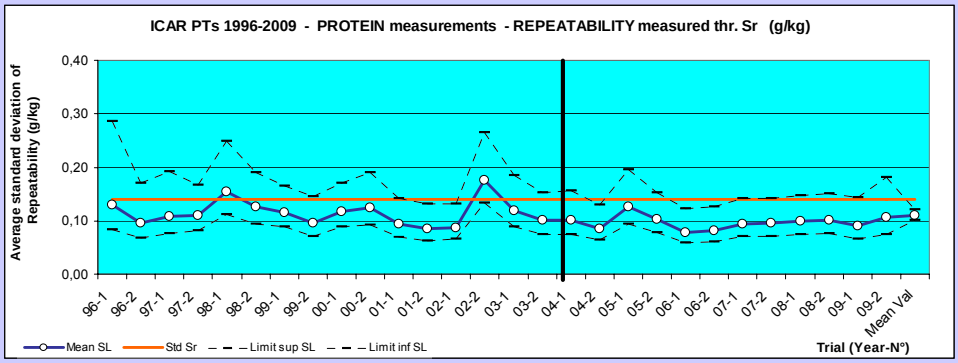
Precision for PROTEIN



Standard sr = 0.14 g/Kg

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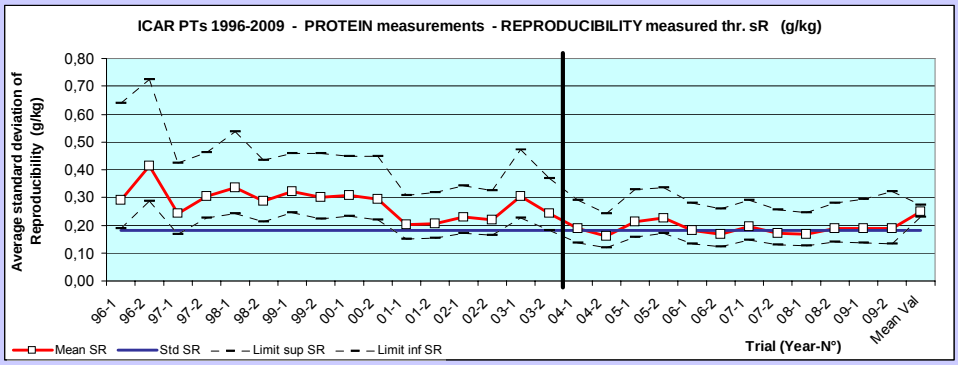
Precision for PROTEIN



Standard sR = 0.18 g/Kg

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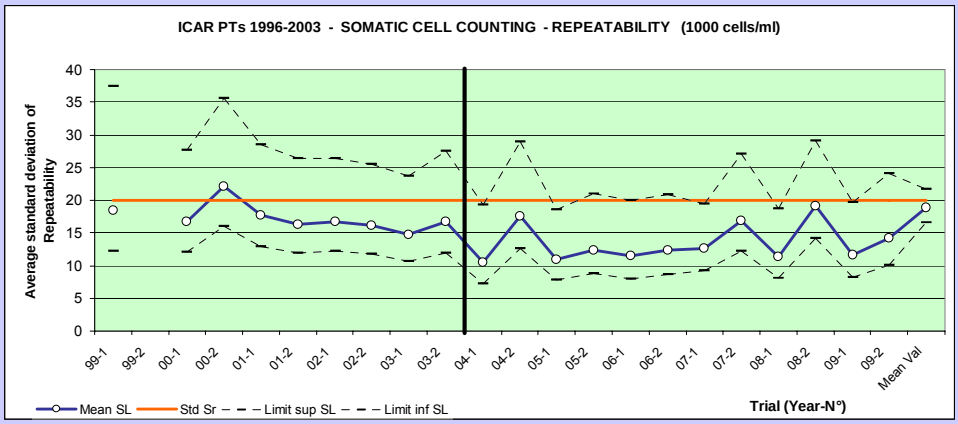
Precision for SOMATIC CELL COUNTING



Standard sr = 20 000cells/ml

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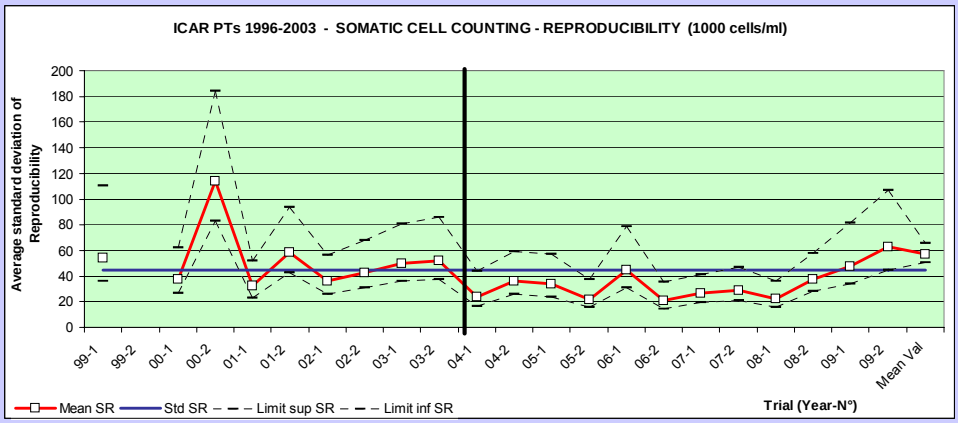
Precision for SOMATIC CELL COUNTING



Standard sR = 45 000cells/ml

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Fat (g/kg)

N° ICAR	1996 - 2009	1996 - 2003	2004 - 2009	2008 - 2009	Limits	Standard
Mean SL	0,09	0,09	0,08	0,07	0,10	0,07
Mean d	-0,01	-0,01	-0,01	0,00	0,20	
Mean Sd	0,16	0,18	0,13	0,13	0,30	
Mean D	0,20	0,23	0,18	0,18	0,36	
Mean SR	0,21	0,24	0,20	0,19	0,37	0,14
Number N	387	203	184	60		

Protein (g/kg)

N° ICAR	1996 - 2009	1996 - 2003	2004 - 2009	2008 - 2009	Limit	Standard
Mean SL	0,11	0,12	0,10	0,10	0,20	0,14
Mean d	-0,05	-0,07	-0,04	-0,04	0,25	
Mean Sd	0,14	0,16	0,13	0,13	0,20	
Mean D	0,23	0,27	0,22	0,22	0,32	
Mean SR	0,25	0,29	0,24	0,23	0,35	0,18
Number N	253	239	142	50		

Somatic cell counting (1000 cells/ml)

N° ICAR	1996 - 2009	1996 - 2003	2004 - 2009	2008 - 2009	Limits	Standard
Mean SL	18,8	17,40	13,7	14,4	36	20
Mean d	-1,6	2,74	-1,4	-1,2	35	
Mean Sd	42,1	44,08	39,4	37,1	35	
Mean D	54,7	55,69	51,2	51,2	49	
Mean SR	57,2	57,88	53,5	50,5	56	45
Number N	254	111	143	50		

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Selection of reference groups of laboratories for the determination of RMs reference values :

Examples of meta-analysis on 4 last trials

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Example of Individual lab performance

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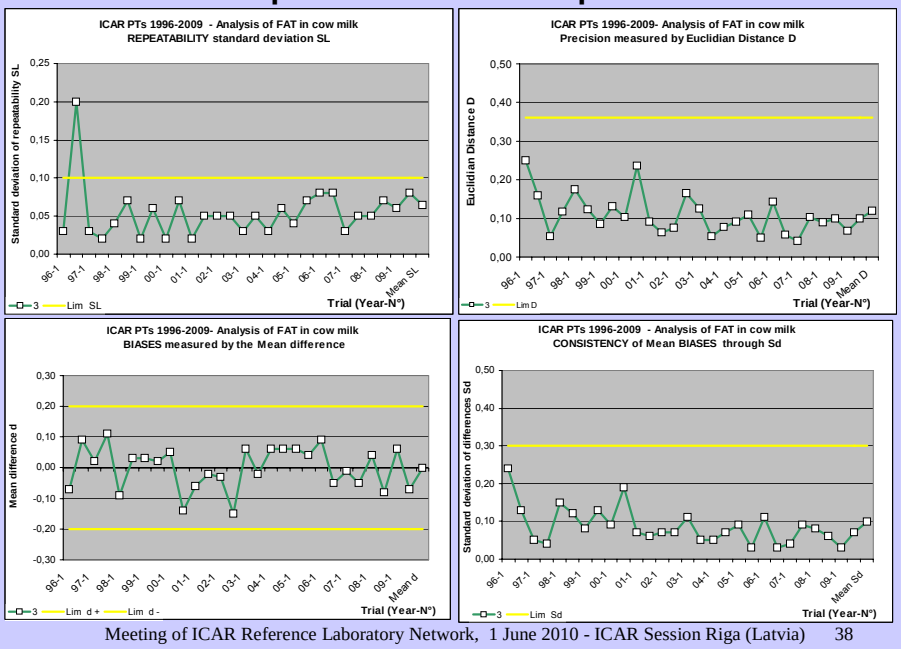
Example of Individual lab performance



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Fat : Ranking to select lab candidate pool to assign reference value (4 last trials)

RANK	Lab ID	TOTAL %	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1		100%	0,04	0,02	0,09	0,05	0,06	0,07
2		75%	0,04	0,05	0,06	0,04	0,06	0,07
3		75%	0,04	0,02	0,08	0,06	0,07	0,07
4		100%	0,04	0,04	0,07	0,05	0,07	0,07
5		50%	0,07	0,01	0,12	0,06	0,07	0,08
6		50%	0,04	0,04	0,05	0,07	0,10	0,10
7		100%	0,07	-0,03	0,06	0,07	0,09	0,10
8		100%	0,07	-0,01	0,06	0,06	0,09	0,10
9		100%	0,05	0,07	0,31	0,08	0,12	0,12
10		50%	0,04	0,11	0,05	0,08	0,14	0,14
11		100%	0,07	-0,11	0,18	0,08	0,15	0,15
12		100%	0,06	-0,05	0,61	0,14	0,16	0,17
13		100%	0,06	0,01	0,18	0,09	0,17	0,18
14		100%	0,12	0,01	0,19	0,16	0,17	0,19
15		25%	0,16	0,18	0,14	0,06	0,19	0,22
16		50%	0,07	-0,09	0,08	0,21	0,23	0,23
17		100%	0,07	-0,16	0,10	0,16	0,24	0,24
18		75%	0,09	0,01	0,18	0,28	0,35	0,36
19		50%	0,11	0,04	0,21	0,35	0,36	0,37
20		100%	0,05	-0,13	0,24	0,12	0,38	0,38
21		25%	2,28	-1,16	0,78	1,81	2,15	2,69

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Fat : Ranking to select lab candidate pool to assign reference value (4 last trials)

RANK	Lab ID	TOTAL %	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1		100%	0,04	0,02	0,09	0,05	0,06	0,07
2		100%	0,04	0,04	0,07	0,05	0,07	0,07
3		100%	0,07	-0,03	0,06	0,07	0,09	0,10
4		100%	0,07	-0,01	0,06	0,06	0,09	0,10
5		100%	0,05	0,07	0,31	0,08	0,12	0,12
6		100%	0,07	-0,11	0,18	0,08	0,15	0,15
7		100%	0,06	-0,05	0,61	0,14	0,16	0,17
8		100%	0,06	0,01	0,18	0,09	0,17	0,18
9		100%	0,12	0,01	0,19	0,16	0,17	0,19
10		100%	0,07	-0,16	0,10	0,16	0,24	0,24
11		100%	0,05	-0,13	0,24	0,12	0,38	0,38
12		75%	0,04	0,05	0,06	0,04	0,06	0,07
13		75%	0,04	0,02	0,08	0,06	0,07	0,07
14		75%	0,09	0,01	0,18	0,28	0,35	0,36
15		50%	0,07	0,01	0,12	0,06	0,07	0,08
16		50%	0,04	0,04	0,05	0,07	0,10	0,10
17		50%	0,04	0,11	0,05	0,08	0,14	0,14
18		50%	0,07	-0,09	0,08	0,21	0,23	0,23
19		50%	0,11	0,04	0,21	0,35	0,36	0,37
20		25%	0,16	0,18	0,14	0,06	0,19	0,22
21		25%	2,28	-1,16	0,78	1,81	2,15	2,69

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Protein : Ranking to select lab candidate pool to assign reference value (4 last trials)

RANK	Lab ID	TOTAL %	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1		100%	0,05	-0,02	0,11	0,08	0,09	0,10
2		50%	0,07	-0,01	0,11	0,07	0,10	0,11
3		25%	0,16	0,08	0,12	0,12	0,00	0,11
4		25%	0,03	-0,02	0,07	0,11	0,12	0,12
5		100%	0,09	0,09	0,09	0,07	0,12	0,13
6		100%	0,06	0,01	0,14	0,08	0,13	0,14
7		75%	0,09	-0,09	0,19	0,08	0,13	0,14
8		100%	0,06	0,04	0,08	0,09	0,14	0,15
9		75%	0,12	0,05	0,14	0,09	0,13	0,16
10		100%	0,05	-0,04	0,20	0,08	0,16	0,16
11		100%	0,16	-0,01	0,09	0,11	0,15	0,18
12		100%	0,10	-0,10	0,36	0,12	0,19	0,21
13		50%	0,10	0,04	0,17	0,22	0,22	0,24
14		75%	0,10	0,16	0,15	0,17	0,23	0,24
15		100%	0,09	-0,12	0,12	0,12	0,23	0,24
16		75%	0,17	-0,20	0,52	0,09	0,25	0,27
17		100%	0,08	-0,28	0,10	0,07	0,31	0,32
18		100%	0,21	-0,20	0,31	0,24	0,39	0,41
19		100%	0,12	-0,30	0,41	0,74	0,87	0,88
20		100%	0,10	-0,39	0,33	1,61	1,73	1,73

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Protein : Ranking to select lab candidate pool to assign reference value (4 last trials)

RANK	Lab ID	TOTAL %	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1		100%	0,05	-0,02	0,11	0,08	0,09	0,10
2		100%	0,09	0,09	0,09	0,07	0,12	0,13
3		100%	0,06	0,01	0,14	0,08	0,13	0,14
4		100%	0,06	0,04	0,08	0,09	0,14	0,15
5		100%	0,05	-0,04	0,20	0,08	0,16	0,16
6		100%	0,16	-0,01	0,09	0,11	0,15	0,18
7		100%	0,10	-0,10	0,36	0,12	0,19	0,21
8		100%	0,09	-0,12	0,12	0,12	0,23	0,24
9		100%	0,08	-0,28	0,10	0,07	0,31	0,32
10		100%	0,21	-0,20	0,31	0,24	0,39	0,41
11		100%	0,12	-0,30	0,41	0,74	0,87	0,88
12		100%	0,10	-0,39	0,33	1,61	1,73	1,73
13		75%	0,09	-0,09	0,19	0,08	0,13	0,14
14		75%	0,12	0,05	0,14	0,09	0,13	0,16
15		75%	0,10	0,16	0,15	0,17	0,23	0,24
16		75%	0,17	-0,20	0,52	0,09	0,25	0,27
17		50%	0,07	-0,01	0,11	0,07	0,10	0,11
18		50%	0,10	0,04	0,17	0,22	0,22	0,24
19		25%	0,16	0,08	0,12	0,12	0,00	0,11
20		25%	0,03	-0,02	0,07	0,11	0,12	0,12

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Somatic cell counting : Ranking to select lab candidate pool to assign reference value (4 last trials)

RANK	Lab ID	TOTAL %	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1		100%	13,4	5,8	12,5	15,5	19,3	21,5
2		25%	12,0	11,0	3,5	17,0	20,2	22,0
3		100%	6,3	-0,3	24,2	14,5	22,6	23,0
4		100%	8,0	-11,3	19,6	14,8	22,3	23,0
5		100%	10,6	-11,5	42,9	18,5	22,8	24,0
6		100%	14,3	10,5	19,8	22,7	29,4	31,1
7		100%	7,0	-2,5	24,2	30,6	34,2	34,5
8		50%	9,6	-22,5	26,6	24,5	36,7	37,4
9		100%	24,6	3,3	35,7	26,8	37,0	40,8
10		75%	19,4	-31,0	100,9	26,8	44,2	46,3
11		75%	11,9	-32,3	17,8	31,5	47,4	48,2
12		50%	10,6	-22,0	20,2	49,4	54,4	54,9
13		25%	7,0	-43,0	41,0	41,0	59,4	59,6
14		100%	13,3	-44,8	36,9	52,6	77,9	78,4
15		100%	22,9	-51,5	27,7	56,5	81,9	83,5
16		100%	16,4	54,5	41,1	57,8	83,6	84,4
17		50%	15,6	-57,5	36,7	76,5	98,1	98,7
18		75%	69,3	-7,0	51,5	85,6	102,8	113,9

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Somatic cell counting : Ranking to select lab candidate pool to assign reference value (4 last trials)

RANK	Lab ID	TOTAL %	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1		100%	13,4	5,8	12,5	15,5	19,3	21,5
2		100%	6,3	-0,3	24,2	14,5	22,6	23,0
3		100%	8,0	-11,3	19,6	14,8	22,3	23,0
4		100%	10,6	-11,5	42,9	18,5	22,8	24,0
5		100%	14,3	10,5	19,8	22,7	29,4	31,1
6		100%	7,0	-2,5	24,2	30,6	34,2	34,5
7		100%	24,6	3,3	35,7	26,8	37,0	40,8
8		100%	13,3	-44,8	36,9	52,6	77,9	78,4
9		100%	22,9	-51,5	27,7	56,5	81,9	83,5
10		100%	16,4	54,5	41,1	57,8	83,6	84,4
11		75%	19,4	-31,0	100,9	26,8	44,2	46,3
12		75%	11,9	-32,3	17,8	31,5	47,4	48,2
13		75%	69,3	-7,0	51,5	85,6	102,8	113,9
14		50%	9,6	-22,5	26,6	24,5	36,7	37,4
15		50%	10,6	-22,0	20,2	49,4	54,4	54,9
16		50%	15,6	-57,5	36,7	76,5	98,1	98,7
17		25%	12,0	11,0	3,5	17,0	20,2	22,0
18		25%	7,0	-43,0	41,0	41,0	59,4	59,6

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Conclusions

Observations between 2004 and 2009 have shown :

1- Improved quality in individual performances between labs,

2- Lower frequency of abnormal scores (outliers):

Outliers noted as resulting from :

- poor sample handling,
- testing disorder,
- data reporting,
- erroneous calculation,
- calibration

Care and GLP in performing tests always needed.

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Conclusions

Observations between 2004 and 2009 have shown :

3- Precision characteristics Sr and SR (after deletion of strong outlying scores) :

a- Significantly improved vs the the former period (1996-2003)

b- Closer to standard values of IDF 1, IDF 20 and IDF 148 :

- Fat : Between IDF 1:1996 and the 2010 revised values
- Protein : Aligned in mostly onto standard values of IDF 20
- SCC : * Mostly in line with IDF 148-2 … but
  * recent trend for higher frequency in calibration defects

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Conclusions

Collection of data between 2004 and 2009 have confirmed the possibility for :

- International anchorage for ICAR countries through capable laboratories operating in international PTs,
- Continuous improvement of lab analytical performance
- Estimation of the overall precision of methods used in a well defined group for collective interest,
- Identification / selection of laboratories to elaborate adequate reference values (international RMs).

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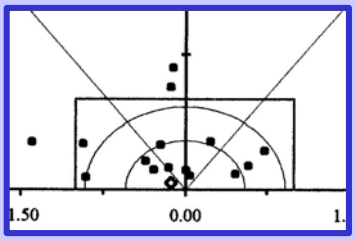

Thank You for your attention !

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International Reference System for Somatic Cell Counting in Milk – A World Wide Challenge

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Abstract

In a globalizing world analytical results play a major role in free and fair trade. However, worldwide equivalence of analytical results can not be ensured by "only" producing standardized analytical methods. For some parameters standardized reference materials are lacking and the reference method shows limited performance. It is there that a reference system should serve to optimally safeguard equivalence.
In this paper it is explained, what a reference system is and why the parameter of Somatic Cell Counting (SCC) was chosen as a first example for implementation and why this represents a typical problem. An outline of a reference system for SCC is drawn and the time plan and the next actions of the joint IDF / ICAR project group are described.

Keywords: reference system, raw milk analysis, somatic cell count, SCC, relation between reference and routine method, joint IDF / ICAR project group

1. What is a Reference System?

A reference system is a systematically developed anchoring system that is fed by different types of information from various sources in a laboratory network structure:

- reference method results;
- routine method results;
- results from proficiency testing.

Joint recognition by regulatory bodies, competent authorities and other stakeholders is essential for an effective functioning. A more extensive explanation on the background and aims as well as on the elements of reference systems was published in the Bulletin of the IDF 427/2008.

2. Why Somatic Cell Counting (SCC) as a First Example?

SCC is one of the most frequently performed tests worldwide, estimated at over 500.000.000 tests/year.

SCC – as an indicator for udder health status – is relevant in food legislation, payment of raw milk and also has a major impact on farm management and breeding programs.

SCC data are obtained almost exclusively by automated high-throughput fluoro-opto-electronic counting instruments, which are calibrated and controlled with more or less defined milk samples giving a "reference level" for counting. This reference level derives in many cases from the application of the reference method, a direct microscopic cell counting according to ISO 13366-1|IDF 148-1.

Somatic Cell Counting as a Typical Problem

Traditional calibration schemes are especially problematic with SCC, because several preconditions are only poorly met. It is necessary to repeat the reference method in more than one lab to arrive at an acceptable precision and accuracy of resulting reference values. Results from a collaborative study carried out in October 2005 show that repeatability r and reproducibility R is rather limited (see also table below). The recently revised ISO 13366-1|IDF 148-1 on the microscopic reference method provides a better description on "what to count and not to count". Still, the reference method is tedious and cumbersome and requires experience and frequent execution in order to safeguard adequate competence of the analyst and a proper counting of the "analyte".

Certified reference material ("golden standard") is not available. Secondary reference materials have problems with shelf life and batch homogeneity during storage. Different matrices and cell types are used for the preparation of these secondary reference materials.

In routinely exercised somatic cell counting, well functioning automated fluoro-opto-electronic methods are used. However, the target analyte of these actual routine methods is not commonly accepted as "reference" basis.

Therefore, a true common basis for the calibration of routine instruments is in fact lacking. As a consequence, several routine laboratories have put their own 'reference system' in place in order to anchor their counting level.

For all these reasons, SCC serves as illustration of a typical problem and makes it into a true candidate parameter for implementing a world wide adopted reference system approach.

ISO 13366|IDF 148, part 1 vs. part 2 (all values in '000/ml) :

	Mean	s_r	sR	r	R
Reference	245	38	41	107	114
	679	69	79	192	218
Routine	245	13	20	36	57
	679	21	40	59	112

To overcome such deficiencies multiplication of the different parts of a traditional calibration scheme can help along. Integration of such multiple calibration models leads to a reference system, which provides a safeguarded SCC level and herewith mutual trust in analytic data.

3. Outline of Reference System for Somatic cell Counting

A reference system is regarded as a tool to provide a commonly acknowledged reference level for a given analytical parameter. It is characterized as a systematically developed anchoring system that is fed by different types of information from various sources, i.e. from reference materials, reference method analysis, routine method results and proficiency testing in a laboratory network structure. Joint recognition by regulatory bodies, competent authorities and other stakeholders is essential for an effective functioning.

A joint project group of IDF and ICAR has recently outlined a reference system for SCC and aims for its implementation during the next years. The system will be fed by routine and reference laboratories to characterize one or more (secondary) reference materials and systematically assign a "true" value to each material. This "assigned value" will represent the anchor level to which local routine laboratories can relate to. A system, well structured and anchored means avoiding fluctuations between different batches of RMs and subsequent calibrations.

The following scheme shows the principle.

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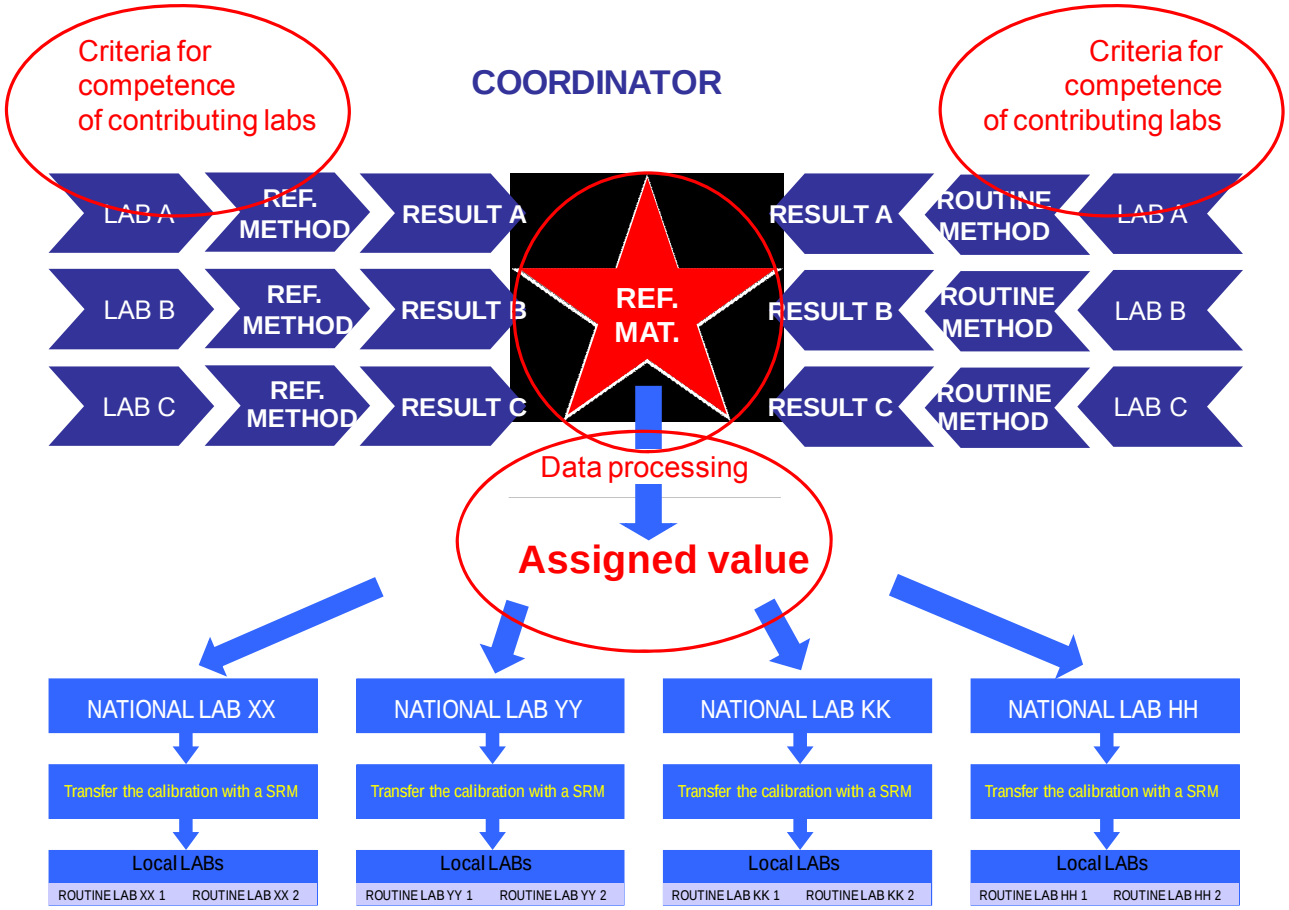


4. What has the project achieved yet ?

In the joint IDF / ICAR project group now 4 continents and 16 countries are represented.

A strategic aspect is to communicate the aim of the project in the right way. This is achieved through meetings, by publishing papers, by publication of a newsletter and creating visibility on the ICAR and IDF websites. The communication will be oriented towards both the analytical stakeholders (labs, RM providers) as well as others (animal health bodies, authorities).

Questionnaires for reference material providers and routine laboratories bring information on how available reference materials produced in several continents and more than 15 countries are used. This information will be useful to draft guidelines for reference materials and to draw a picture of the interlinkages between the different existing local analytical systems.

The first calculation models for assessing both proficiency testing schemes and the performance therein of laboratories involved with the assignment of reference values are under development within a group of statistical experts.

5. Next actions

The project group has identified the important parts to come to a reference system and is working out the details of a pilot model, which is to be evaluated in practise.

Crucial parts of this pilot model will be (amongst others):

a suitable reference material (representative and stable);

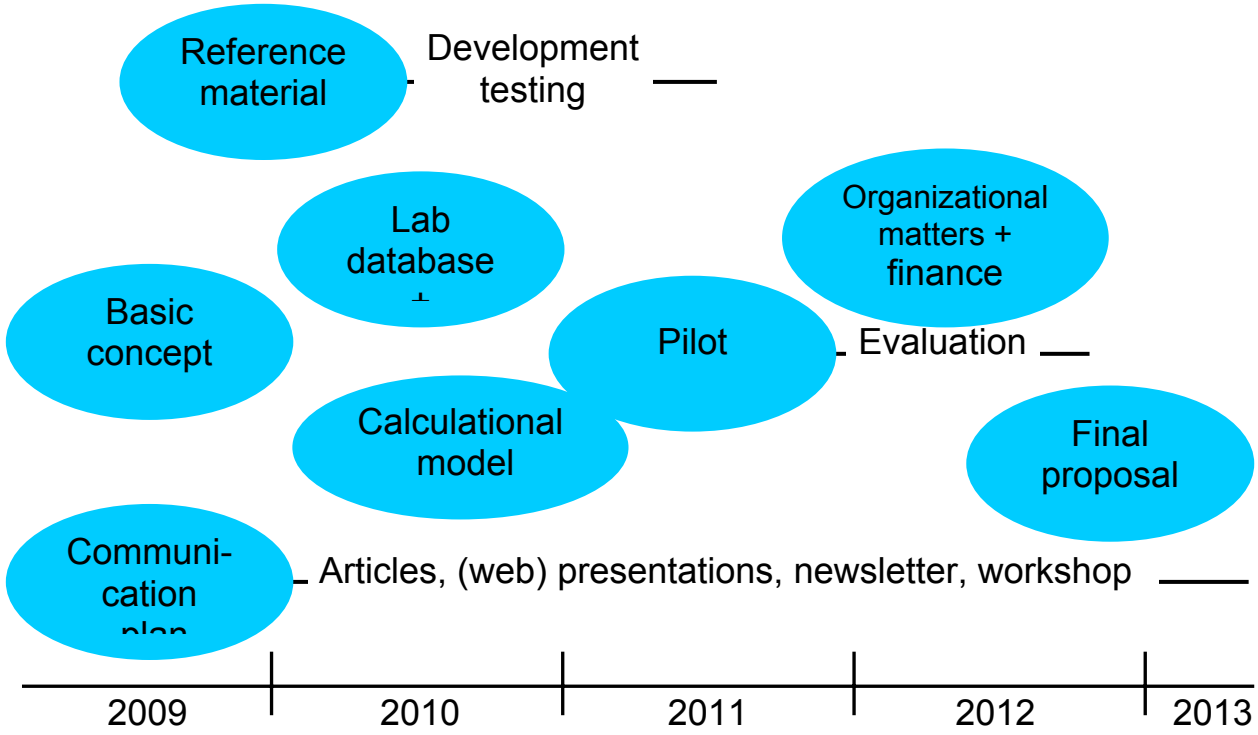
a laboratory database with a system for competence scoring;

a calculation model for determining optimized assigned values;

The project group follows a bottom–up approach. This means to collect and make use of existing and functioning local structures.

Of course some organizational matters and financial issues have to be addressed in the near future, too.

Time Plan



6. Conclusions

Somatic cell count in milk is an excellent parameter to explore the feasibility of a reference system approach. It is a very relevant parameter in food legislation, in farm management and in animal breeding and the reference method has distinct drawbacks.

The world wide challenge of this innovative analytical approach is to create mutual trust between the actors involved and to share useful data and experience which are obtained in daily analytical life.

A collaborative atmosphere at the national and international levels will help – and will be needed – to complete the puzzle of this sophisticated but also more robust analytical approach that is focussed at obtaining better analytical equivalence.

A robust reference system should produce results, which are valid in a three-dimensional scale: worldwide, over time and between different methods. ICAR and IDF as dedicated international organisations have taken up the glove to develop and implement this innovative and valuable approach.

International Reference System for Somatic Cell Counting in Milk

A World Wide Challenge

Christian Baumgartner

on behalf of the

IDF/ICAR Project Group on
Reference System for Somatic Cell Counting in Milk

IDF/ICAR Project Group (April.2010)

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- Christian Baumgartner, Milchprüfring Baye
- Thomas Berger, Agroscope Liebefeld-Posi
- **Harrie van den Bijgaart**, Qlip (NL)
- Ute Braun, muva (DE)
- Pierre Broutin, Bentley Instruments SARL
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- Paul Jamieson, SAITL (NZ)
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- Tiina Putkonen, Finnish Food Safety Authority Evira (FI)
- Dalia Riaukiene, Pieno Tyrimai (LT)
- Andrea Rosati, ICAR Secretariat (IT)

3 continents
14 countries
10 male – 10 female

IDF and ICAR

……you know!

IDF and ICAR

www.fil-idf.org

International Dairy Federation

IDF is the pre-eminent source of scientific and technical expertise for all stakeholders of the dairy chain. Membership covers 56 countries and is growing. IDF accounts for about **86%** of current total milk production worldwide.

The mission of IDF is *to represent the dairy sector worldwide by providing the best global source of scientific expertise and knowledge in support of the development and promotion of quality milk and dairy products to deliver consumers with nutrition, health and well-being.*

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SOUTH AFRICA (ZA) ZIMBABWE (ZW)

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Goal of IDF/ICAR cooperation?

international

Standardization

➔ Equivalence

Not only setting standards, but trying to ensure that standards are kept!!!

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Why Analytical Standardization?

When goods are moving, analytical results should (must) be comparable and "equivalent"

- worldwide
- on the long run
- between different methods

➔ **Equivalence… anywhere – anytime – anyhow**

But what „Normal" / Reference to relate to? ⇨ **equivalent and correct / "true"**

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Reference Methods

- Reference methods serve as **anchor** (examples):
  - defining methods like Codex Type I methods
    - Milk – Protein: ISO 8968-1|IDF 20-1 (Kjeldahl titrimetry)
    - Cheese – Moisture: ISO 5534|IDF 4 (gravimetry)
  - designated methods like Codex Type II methods
    - Butter – Salt: ISO 1738|IDF 12 (titrimetry)
    - Whey powder – Lactose: IDF 79B (enzymatic)

But Reference Methods are too costly and time consuming for daily work…

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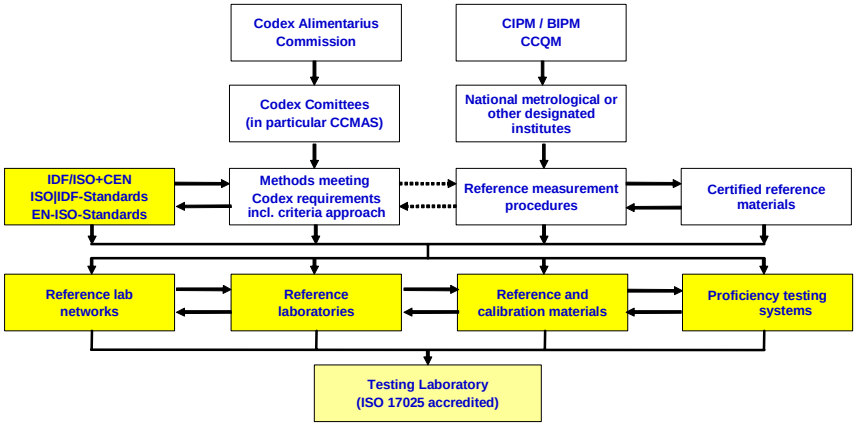
Routine Methods

- High throughput, often automated
- High precision
- User friendly
- Immediate availability of data
- Low labour, low cost per analysis
- Crucial for the functioning of "daily dairying life"

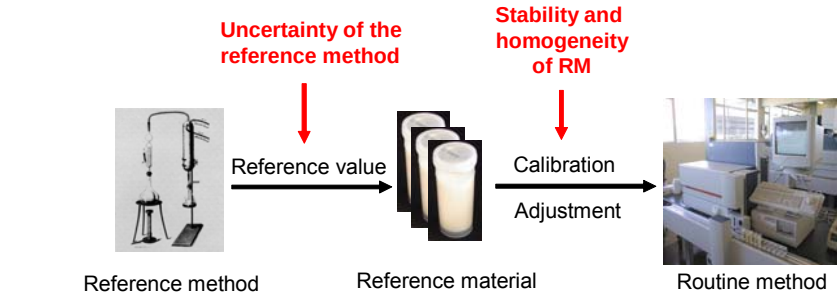
Traceability to **defined units** is key for a coherent expression and use of results!

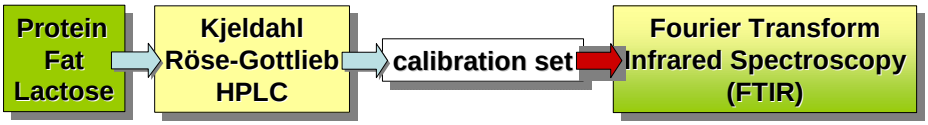
Metrologically Traceable Measurement System for Food Analysis (CIPM/BIPM)



Overall goal ➔ Comparable, traceable measurement results with stated uncertainty, providing reliable data for decisions in trade, regulation and risk assessment.

The ´Traditional´ Approach





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…and the ´Real Life´ Situation

- Reference methods are indispensable, but…
  - often not suitable for large scale routine application
  - with some important practical applications the precision is not satisfactory
  - no guarantee for a reliable reference when applied in only one laboratory
- Solutions cannot always be achieved by straightforward analytical means or reference materials
- Implementing a **reference system** is a complementary option to safeguard “equivalence”

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What is a Reference System?

- Well-structured anchoring **system** fed by different types of information
  - reference method
  - routine method results
  - proficiency testing results

from many different sources

- Traceable competence as prerequisite
- Recognition/adoption by regulatory bodies, competent authorities

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For further information...

Bulletin of the International Dairy Federation 427/2008 Towards a Reference System for Somatic Cell Counting in Milk

2. Architecture of reference systems, status quo of Somatic Cell Counting and concept for the implementation of a reference system for Somatic Cell Counting

C. Baumgartner[1]

Summary

The definition of "reference" relates to two meanings. One relates to "tes
or "certification", the other to the aspect of "information", "evidence" o
are a good description of the purpose of reference systems, which are
and improve the traditional way of calibration of routine methods.

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Why SCC as a First Example?

- SCC is one of the most frequently performed tests worldwide (~ 500.000.000 tests/year)
- SCC – as an indicator for udder health status – is relevant in food legislation, payment of raw milk and also has a major impact on farm management and breeding programs
- Farm management, breeding programs
  ➔ economics!

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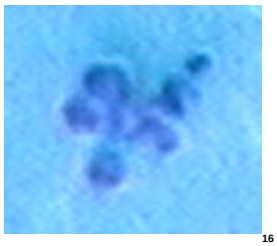
SCC as a Typical Problem

- No clear definition what to analyze; i.e. the analyte is defined by the traditional microscopic reference method;
- The reference method derives from “historic” ages;
- The reference method is tedious, cumbersome and has poor performance;
- “Target analyte” of nowadays´ routine methods is not commonly accepted as new “reference” basis;
- No CRM/“golden standard” available;
- SRMs have problems with shelf life and batch homogeneity during storage;



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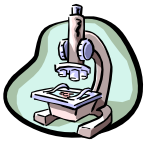
Somatic Cell Counting in Milk

Reference method



Direct microscopic somatic cell count

Routine methods

Somascope™

Somacount™

Nucleocounter™

DCC Counter™

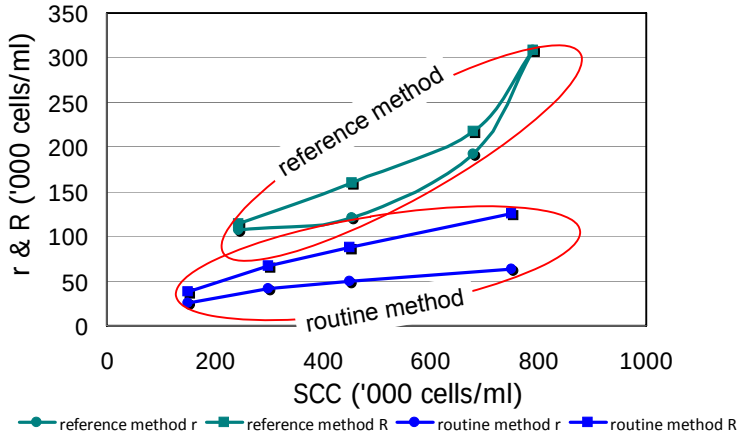
Fossomatic™

Precision Reference vs. Routine

- ISO 13366|IDF 148, part 1 vs. part 2
- All SCC values in '000/ml:

	Mean	s_r	sR	r	R
Reference	245	38	41	107	114
	679	69	79	192	218
Routine	245	13	20	36	57
	679	21	40	59	112

Precision Reference vs. Routine



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How to begin?

Building Blocks for a Reference System

- Standards: ISO 13366|IDF 148 parts 1-2 (2008)
- Willingness to cooperate in a laboratory network
- Reference materials
- Proficiency testing schemes
- Training course system
- Gathering all data and create a data base

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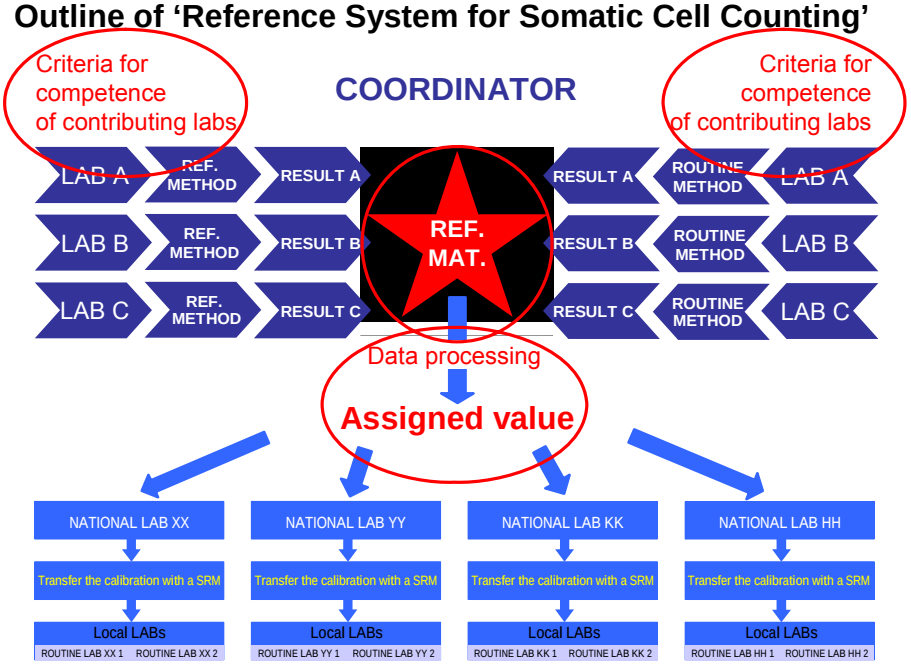
Outline of 'Reference System for Somatic Cell Counting'



Criteria for Reference Materials

- Range (cow, goat, sheep)
- Representativeness (matrix, cell material, preservation)
- Adequately assigned values
- Homogeneity
- Stability during shipment, storage and pretreatment
- Validated as being 'fit for purpose'

➔ RM to be selected and optimized

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Traceable Competence of Labs

- Level of analytical quality assurance
- Participation and performance in proficiency testing
- Recording and scoring of performance in laboratory database

➔ scoring system to be developed

Calculation Model

Arriving at assigned values based on:

- reference method results
- routine method results
- data processing model with applying weighing factors based on traceable competence of contributing labs

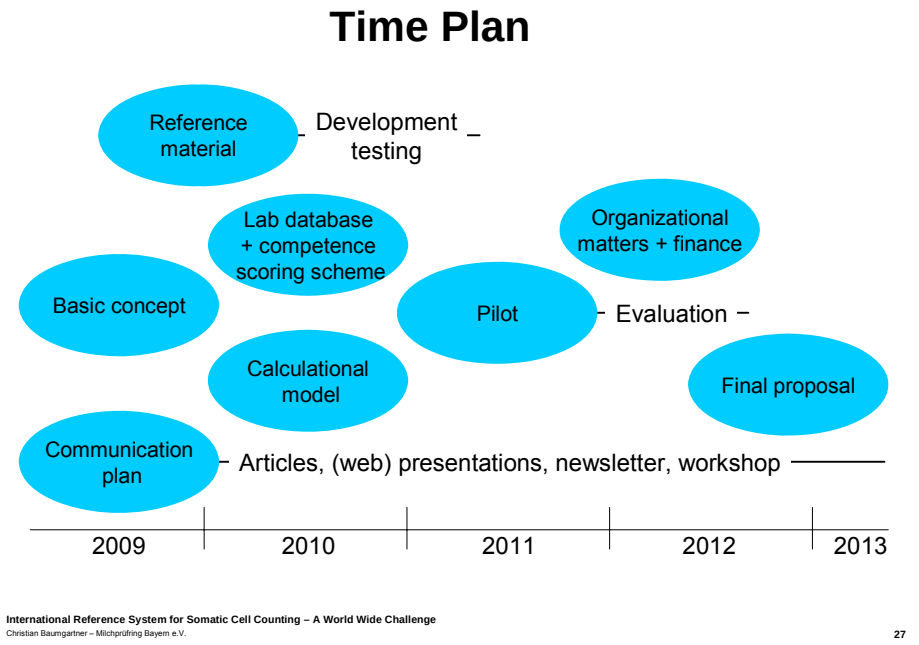
➔ calculational model to be developed

Next Actions (1)

- Arriving at suitable reference material
  - Contact with reference material suppliers
  - Questionnaire on applied reference materials
  - Selection/optimization
- Laboratory database
  (incl. system for scoring competence)
- Outline of calculation model

Next Actions (2)

- Communication plan about the project
  - Analytical-oriented stakeholders (labs, RM providers)
  - Other stakeholders (animal health bodies, authorities)
- Ordering thoughts about
  - Training course system
  - Coordinator (position, competence, tasks)
  - Finance


Time Plan
Reference material
Development testing
Lab database + competence scoring scheme
Organizational matters + finance
Basic concept
Pilot
Evaluation
Calculational model
Final proposal
Communication plan
Articles, (web) presentations, newsletter, workshop
2009
2010
2011
2012
2013
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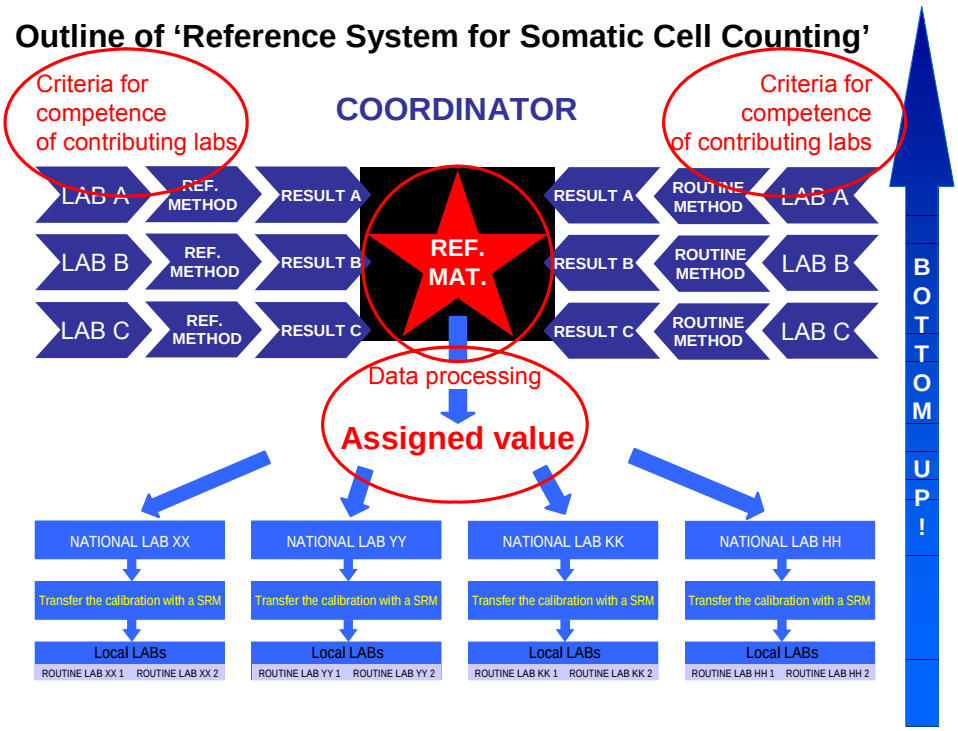
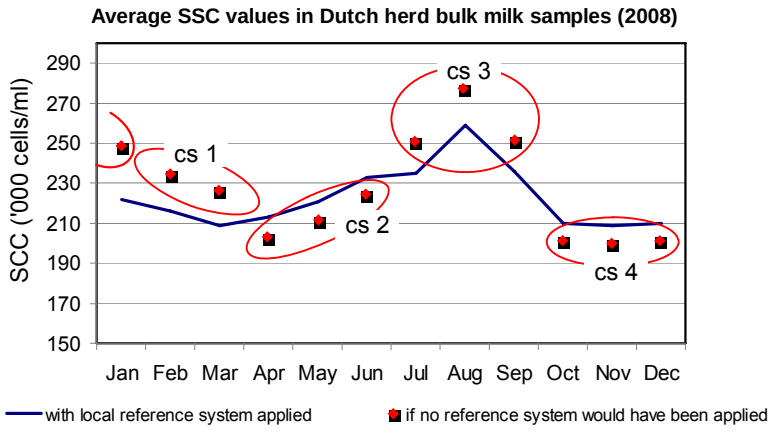

Outline of 'Reference System for Somatic Cell Counting'
Criteria for competence of contributing labs
COORDINATOR
Criteria for competence of contributing labs
LAB A
REF. METHOD
RESULT A
REF. MAT.
RESULT A
ROUTINE METHOD
LAB A
LAB B
REF. METHOD
RESULT B
RESULT B
ROUTINE METHOD
LAB B
LAB C
REF. METHOD
RESULT C
RESULT C
ROUTINE METHOD
LAB C
Data processing
Assigned value
BOTTOM UP!
NATIONAL LAB XX
NATIONAL LAB YY
NATIONAL LAB KK
NATIONAL LAB HH
Transfer the calibration with a SRM
Local LABs
ROUTINE LAB XX 1
ROUTINE LAB XX 2
ROUTINE LAB YY 1
ROUTINE LAB YY 2
ROUTINE LAB KK 1
ROUTINE LAB KK 2
ROUTINE LAB HH 1
ROUTINE LAB HH 2

Local Example from NL



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cs = calibration set 29

Conclusions

- Reference systems serve to complement the 'traditional' way of calibration of routine methods for safeguarding the validity of analytical results.
- A wider implementation of recognized reference systems will improve the acceptance and mutual confidence in analytical results.
- Somatic cell counting is an excellent parameter to explore the feasibility of a world-wide functioning reference system, thereby demonstrating the potential benefit of an implementation for other parameters.

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A Sisyphus job… maybe…

...but with a little help from our friends?!

Latvian milk recording analysis and Dairy Laboratory Ltd. in the ICAR analytical reference system

Diana Ruska
SIA Piensaimnieku Laboratorija, Ulbroka (Riga), Latvia

Abstract

Dairy farming is one of the basic agricultural sectors in Latvia. Therefore milk recording system is very important for our farmers and it has very old history. Initially in Latvia farmers could test milk recording analyses in every milk laboratory. Later when centralisation of milk recording system started farmers could do milk recording analyses only in laboratory of the Breeding station. Now we are working with Agricultural Data Centre program for milk recording and farmers can do these analyses only in the accredited laboratory. Breeding system in Latvia is regulated by the government. The state reference laboratory is the supervisor all of Latvian milk control laboratories. The reference laboratory organizes proficiency testing and prepares reference materials for fat, protein content and somatic cells count.

Dairy Laboratory Ltd. works in the both fields of milk analysing, such as milk recording analysis and payment analysis. The quality system in the laboratory is very strictly determined according ISO 17025 standard. We are working with ISO, IDF and validated methods. Scopes of accreditation are raw milk physical - chemical testing and milk, milk products and water microbiology. We work with instrumental methods and reference methods for milk microbiology and for determinate freezing point in milk. Our laboratory takes part in the different proficiency testing in Latvia and in the reference laboratories of European countries. Operation with different reference materials gives reliable, traceable and very precise results of analyses.

Staffs of the laboratory for a whole time get new experience and knowledge in the testing field. As well as we organise training for our customers in milk sampling system, transporting and milk recording system.

Keywords: national regulation, sampling, accreditation

1. Milk recording in Latvia

First information about milk recording analyses for milk fat content and milk yield in Latvia we can find in beginning of 20th century. Total protein for milk recording start analysed in 1980, for somatic cells count in 1998. In 1997 was founded State Agency Agricultural Data Centre (S/A ADC) and was started digital era of milk recording.

Now under milk recording are 120 800 cows in 8062 farms. Every year from 1991 we observe decreasing in number of cows and herds. Situation with milk yield is different and we see increasing in milk recording results and in statistical every year.

2. Latvian milk recording analysis

Latvian milk recording system is voluntary, but we have strong National regulation and support from government for this system. In National regulation are defined requirements for milk testing laboratory, milk sampling, milk analysing and analysed results recording.

The herd owners in whose herd's milk monitoring is being carried out shall receive control forms and reporting lists. All the cows and heifers of the herd, which are older than 24 months, must be indicated in the control forms. Taking milk samples for analysis, controller or the herd owner fills in the control list and sends it together with the milk samples to the Laboratory for processing.

Milk testing laboratory need to have Quality Assurance System according to standard LVS EN ISO/IEC 17025:2005. Accredited at Latvian National Accreditation Bureau (LATAK), Member of European Co-operation of Accreditation.

Milk laboratory for milk recording analysing provide milk samples containers with samples vials volume up to 45 ml, accompanying document for samples, milk samples preservative (BSM II) and transportation of samples.

After testing Laboratory send samples testing results to central data base in Agricultural Data Centre. From data base farmers and breeding specialists take all milk recording information and analyses results.

3. National reference laboratory

The reference laboratory perform following tasks: co-ordinating of activities of the laboratories whose task is to conduct analyses to check the chemical and bacteriological standards; supervision and control of laboratories involved in raw milk control; preparation of calibration samples using reference methods, twice per month (fat, protein, dry matter, somatic cells count); preparation and implementation of proficiency tests four times per year.

In Latvia are 6 accredited raw milk routine laboratories. Three of them are Milk factory laboratories, they work only for payment testing. Two laboratories are DHI (Dairy Herd Improvement) laboratories in the Breeding and Artificial Insemination Station. One is independent laboratory - Dairy laboratory Ltd. it works to both systems - payment and DHI testing.

4. Dairy laboratory Ltd.

Dairy Laboratory place in milk recording system are in third place. First level is legalisation level from Government and according ICAR Guidelines. Second level are audit level from Food and veterinary service, National reference laboratory, Accreditation bureau – LATAK, Agricultural data centre. Third level is Laboratory and basic of this system are Farmers and Dairies.

Dairy Laboratory is a private company. Our owners are non governmental organisations, stock companies and State. It represents three big groups of interest Farmers, Dairies and Breeding organisations.

Organization structure of Laboratory is following: board (5), administration (2), head of laboratory and quality system (1), technical manager, chemist (1), microbiologist (1), instrumental equipment operators (2), data operators (2) and samples collection (2).

Staff of Laboratory every time renew skills and competence in testing field. Participation in specialised course for staff, study in High school. Technical tours in other testing laboratories (The Netherlands, Germany, Cyprus, Estonia, Lithuania).

For equipment calibration in laboratory are used Reference materials RM from different producers: Latvia, France, Germany, Denmark, Italy and USA.

Laboratory regular take part in several International Proficiency Testing schemes in Latvia and in Europe countries for each parameter at least one time per year. In Latvia – 4 times per year, in Germany – 5 times per year and France, Italy, England – 1 times per year.

Our laboratory has following testing scope for milk compounds (Fat, Protein, Lactose, Casein, Urea content and Total solids), milk quality (Somatic cells count, Total bacteria count, pH) and milk falsification (Inhibitor, Freezing point).

All testing methods, what we use in Laboratory are based on National, international and in house validate methods. For milk compound we work with Infra red testing methods. In Laboratory we have two reference methods for bacteria counting and freezing point.

Basic place of our works is precision of testing results, therefore important to give testing knowledge for our customers. For them we organise education courses and giving individual consulting in milk recording system, milk sampling for milk recording, milk sampling for payment analyzing and consulting about calibration of equipment.

We active take part in several work groups and projects in testing field and we have very close relationship with Ministry of Agriculture, Agricultural Data Centre, Food and veterinary department, Latvian University of Agriculture, International Dairy Federation (National secretary) and ICAR. Dairy laboratory represents: Quality, Speed and Customer service.

History of milk recording in Latvia

- ✓ First information about milk recording analyses for milk fat content and milk yield we can find in beginning of 20th century
- ✓ Total protein for milk recording start analysed in 1980, somatic cells count in 1998
- ✓ In 1997 was founded State Agency Agricultural Data Centre (S/A ADC) and was started digital era of milk recording

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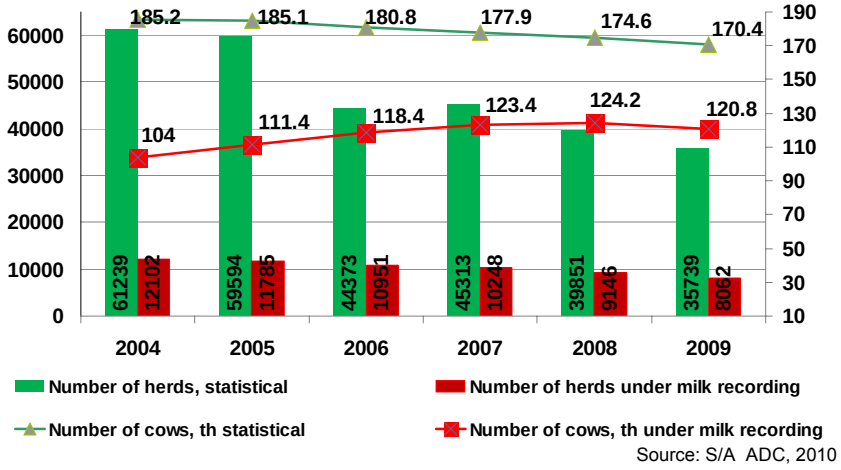
Number of herds and cows under milk recording and statistical



Source: S/A ADC, 2010

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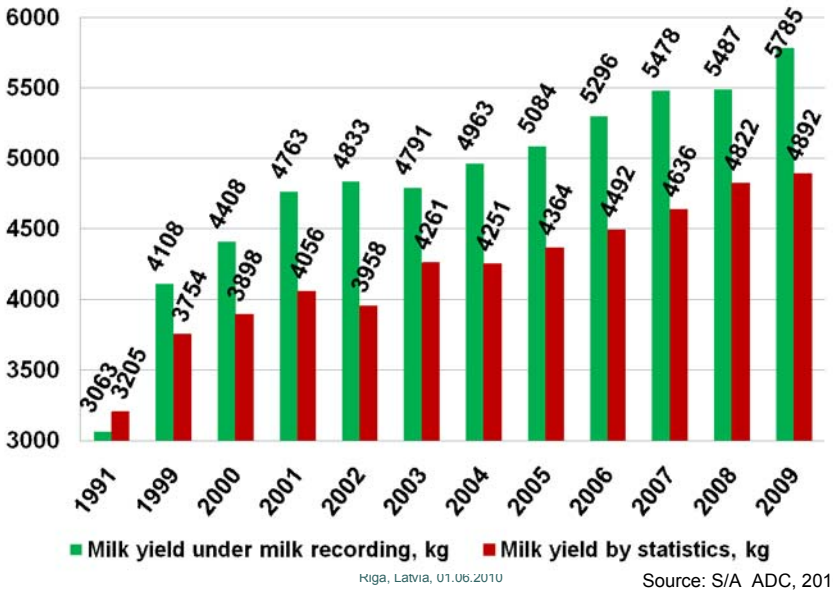
Milk yield under milk recording and statistical



Source: S/A ADC, 2010

Latvian milk recording analysis

- ✓ Milk recording system in Latvia is voluntary
- ✓ In National regulation are defined requirements for:
  - milk testing laboratory
  - milk sampling
  - milk analysing
  - analysing result recording

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Breeding work rule

- ✓ The herd owners in whose herd's milk monitoring is being carried out shall receive control forms and reporting lists
- ✓ All the cows and heifers of the herd, which are older than 24 months, must be indicated in the control forms
- ✓ Taking milk samples for analysis, controller or the herd owner fills in the control list and sends it together with the milk samples to the Laboratory for processing

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Milk testing laboratory

- ✓ Quality Assurance System according to standard LVS EN ISO/IEC 17025:2005

Accredited at:

- ✓ Latvian National Accreditation Bureau (LATAK), Member of European Co-operation of Accreditation

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Milk laboratory provide

- ✓ Milk samples containers with samples vials volume up to 45 ml
- ✓ Accompanying document for samples
- ✓ Milk samples preservative (BSM II)
- ✓ Transportation of samples

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Results recording

- ✓ Samples testing results send to central data base in Agricultural Data Centre
- ✓ From data base farmers and breeding specialists take all milk recording information and analyses results

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National reference laboratory

The reference laboratory perform following tasks:

- co-ordinating of activities of the laboratories whose task is to conduct analyses to check the chemical and bacteriological standards
- supervision and control of laboratories involved in raw milk control
- preparation of calibration samples using reference methods, twice per month (fat, protein, dry matter, somatic cells count)
- preparation and implementation of proficiency tests four times per year

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In Latvia are 6 accredited raw milk routine laboratories:

- ✓ Tree of them are Milk factory laboratories, they work only for payment testing
- ✓ Two laboratories are DHI (Dairy Herd Improvement) laboratories in the Breeding and Artificial Insemination Station
- ✓ One is independent laboratory - Dairy laboratory Ltd. It works to both systems - payment and DHI testing

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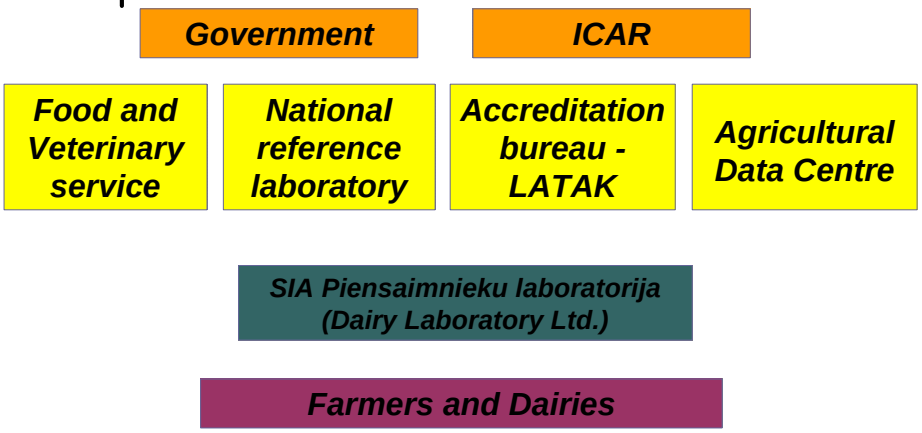
Dairy Laboratory Ltd. place in milk recording system



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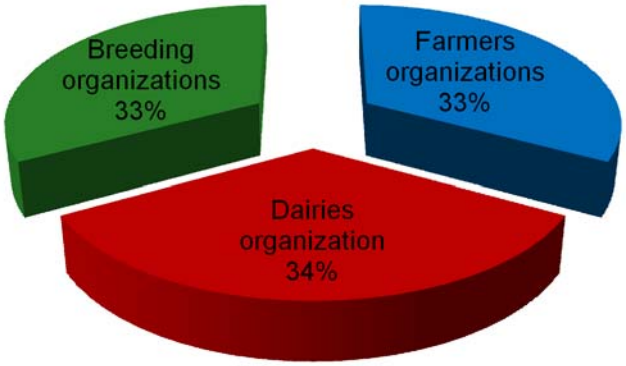
Owners of Dairy Laboratory



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Organization structure of Laboratory

- ✓ Board (5)
- ✓ Administration (2)
- ✓ Head of laboratory and quality system (1)
- ✓ Technical manager, chemist (1)
- ✓ Microbiologist (1)
- ✓ Instrumental equipment operators (2)
- ✓ Data operators (2)
- ✓ Samples collection (2)

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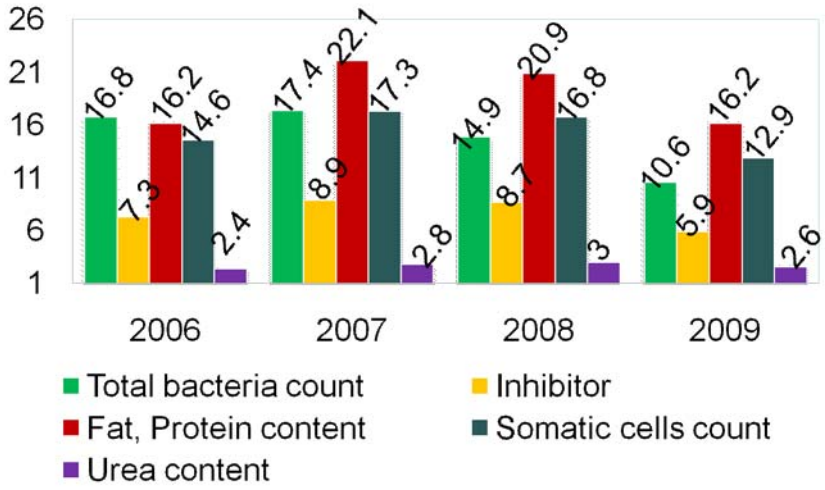
Testing samples quantity, th



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Education of Laboratory personal

- ✓ Staff of Laboratory every time renew skills and competence in testing field:
  - Participation in specialised course for staff
  - Study in High school
  - Technical tours in other testing laboratories (The Netherlands, Germany, Cyprus, Estonia, Lithuania)

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Reference materials (RM)

✓For equipment calibration in laboratory are used RM from different producers:

- Latvia
- France
- Germany
- Denmark
- Italy
- USA

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International Proficiency Testing (PT)

✓Laboratory regular take part in several PT schemes in Latvia and in Europe countries for each parameter at least one time per year

- Latvia – 4 times per year
- Germany – 5 times per year
- France, Italy, England – 1 times per year

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Testing scope

- ✓ Milk compounds
  - Fat content
  - Protein content
  - Lactose content
  - Casein content
  - Urea content
  - Total solids

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Testing scope

- ✓ Milk quality
  - Somatic cells count
  - Total bacteria count
  - pH
- ✓ Milk falsification
  - Inhibitor
  - Freezing point

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One sample 35-40ml
A lot of results

- ✓ *Total bacteria count*
- ✓ *Inhibitor test*
- ✓ *Fat and Protein content*
- ✓ *Somatic Cells count*
- ✓ *Freezing point*
- ✓ *Urea content*
- ✓ *Casein content*
- ✓ *Lactose content*
- ✓ *pH*

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Testing methods

CombiFoss FC - MilkoScan FT 6000, Fossomatic FC

Methods	***Quality parameter***
ISO 9622:1999	Fat, Protein and Lactose content
LVS EN ISO 13366-2:2007	Somatic cells count
Validated methods	Urea, Casein and Total solids content
	Freezing point
	pH testing

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Testing methods

Methods	Quality parameter	Equipment
LVS EN ISO 4833:2003, MET 001 - Validated	Total bacteria count	Plate count technique BactoScan FC
ISO 5764/IDF 108:2009	Freezing point	Multisample Cryscope 4C3
LVS 174:1999	Inhibitor test	“Delvotest SP”, Accelerator

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Customers education

- ✓ Milk recording system
- ✓ Milk sampling for milk recording
- ✓ Milk sampling for payment analyzing
- ✓ Consulting about calibration of equipment

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Relationship

- ✓ Ministry of Agriculture
- ✓ Agricultural Data Centre
- ✓ Food and veterinary department
- ✓ Latvian University of Agriculture
- ✓ International Dairy Federation (National secretary)
- ✓ ICAR

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International Strategies and New Developments in Milk Analysis

Part 2 – New parameters of interest for milk recording

Determination of fatty acid composition in milk of individual animals by Fourier-Transform Mid Infrared Spectrometry

Marion Ferrand

Institut de l'Elevage, Paris, France

Abstract

The increasing social demand for healthy products leads more and more dairy companies to select collected herd milks according to the fine milkfat composition and could also lead to the introduction of the composition in fatty acid (FA) as a criterion for milk payment. However, today, there is neither rapid method officially validated in France to determine milk fatty acid composition in routine analysis nor tools to allow adaptation of the fine milkfat composition to the evolving consumers demand.

Consequently it has become today of a major interest to define technical levers that will allow milk producers to orient milk fatty acid profiles as soon as the production stage on-farm. Since then the objectives to measure elementary milk components with sufficient accuracy and to identify the genetic and environmental factors affecting the composition are being pursued through a R&D project, initiated in France in 2008, called PhenoFinLait.

The first step was to develop a cheap and large scale phenotyping system for determination of FA individual milk content. A set of equations was developed to estimate fine FA milk composition from MIR (Mid Infra-Red) spectra usually obtained by milk recording laboratory. For several FA, a variable selection was applied to improve the equations. In the end, 15 to 20 FA are well estimated in the three ruminant species (cow, sheep and goat). Statistical research is ongoing to improve estimation for other FA and normalize this method.

This study is part of PhenoFinlait project funded by Apis-Gène, CASDAR, CNIEL, FranceAgriMer, France Génétique Elevage, French Ministry of Agriculture and French Ministry of Research.

Keywords: cow, ewe, goat, milk, fatty acid, mid-infrared (MIR) spectrometry, genetic algorithms, Partial Least Squares (PLS) regression

1. Materiel and methods

1.1 Milk samples

1.1.1 Cow milk samples

First, 154 milk samples from 77 crossbred Holstein X Normande dairy cows were collected in 2008 at the experimental Pin-Au-Haras INRA farm. The cows were a part of a QTL (Quantitative Trait Loci) detection experiment, and they arose from a cross over two generations (F2) between Normande and Holstein breeds that display numerous differences in particular for milk fat and protein contents.

Milk samples were collected twice, in winter and in summer to take into account the possible effect of feed. Cows were in an average stage of lactation of 160 days (77-235) in winter, and 209 days (126-284) in summer. Each time and for each cow, two milk samples were realized during the morning milking. One was analyzed freshly using MIR spectrometry, the other was frozen at -20°C and analyzed using gas chromatography. Finally, on 154 samples, 150 milks were kept in the study due to missing data.

Secondly, 153 milk samples from 54 Montbéliarde and 42 Prim'Holstein were collected in 2009 at the experimental Mirecourt INRA farm. Depending on calving date, the cows belong either to a grazing system or to a mixed crop dairy system. Milk samples were collected using the same protocol as above, but only a part of the cows were present at both sampling. On 153 samples, 100 milks were analysed by gas chromatography.

1.1.2 Goat milk samples

705 milk samples from 235 Alpine dairy goats were collected in 2008 at the INRA experimental farm of Bourges at three stage of lactation (about 40, 150 and 240 days). The goat's diet was almost similar throughout lactation and was based on grass hay offered ad libitum and a commercial concentrate mixture. These samples were collected in tubes containing a preservative (Bronopol).

For each goat, one sample was analysed by MIR spectrometry, and one other was frozen at -20°C. Among them, 149 samples (about 50 per stage of lactation) with a large variability of spectra were selected to be analysed for milk fatty acid composition by the referenced method.

1.1.3 Ewe milk samples

A first sampling was carried out twice in 2008 in the experimental La Fage INRA farm: milk samples were collected from Lacaune dairy ewes, respectively on March 2008 at 80 days in milk (DIM) for 490 ewes in winter diet (hay, silage and concentrates), and on May 2008 at 152 DIM on average for 493 ewes in spring diet including pastures. At each sampling carried out at the morning milking, 2 milk samples were collected, the first fresh one to be analyzed without delay to provide MIR spectra and the second one to be frozen at-20°C for a possible reference gas chromatography carried out later. Accounting for somatic cell count, fat content and milk spectra, 75 milk samples were chosen within each sampling period, i.e a total of 150 frozen milk samples to be analyzed by gas chromatography.

A second sampling, using the same design described above, was performed in 2009 in 3 private flocks, the first one composed of Basco-Bearnaise (BB) ewes, and the two other of Manech red faced (MRF) ewes: a total of 103 milk samples, respectively 35 from BB ewes and 78 from MRF ewes, were collected by the end of April 2009 at 120 DIM on average in pasture diet condition. Accounting for fat content, milk spectra and breed, 50 milk samples were chosen to be analyzed by gas chromatography (respectively 20 and 30 for BB and MRF breed).

Finally 200 milks from Lacaune ewes (150 samplings) or from BB or MRF ewes (50 samplings) with both milk spectra and gas chromatography results were included in the present analysis carried out in dairy sheep.

1.2 MIR spectra

After a transport at 4°C to the laboratory (LILANO of St Lo, LILCO of Surgères and LIAL of Aurillac), fresh milk samples were analyzed for milk spectra extraction using MIR spectrometry with defined routine FT-MIR analyzers (Milkoscan FT6000, Foss and Bentley FTS). Spectra have been recorded from 5012 to 926 cm-1. According to Foss (1998), only informative wavelength bands, i.e. bands not spoiled by water molecule, were kept (representing a total of 446 wavelengths). No pre-treatments were applied as suggested by Soyeurt and al. (2006).

1.3 Fatty acid composition

Frozen milk samples were analyzed for milk fatty acid composition using gas chromatography according to ISO standards (Kramer, 1997). Quantities of 64 fatty acids were expressed in g/100mL. Outliers were removed by Grubb's test as indicated in the norm ISO 8196.

1.4 Calculation of calibration equations

MIR spectra and milk fatty acid composition of samples presenting a large variability in their composition were retained to calculate the equations. For cow and ewe milk, the samples were divided into calibration and validation sets (cow milk: $n_{calibration}$=175 and $n_{validation}$=75, ewe milk: $n_{calibration}$=140 and $n_{validation}$=60).

The equations were developed by univariate and multivariate PLS regression (Tennehaus, 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 ($RMSEP_{cv}$). To improve equations and quality of estimation, a selection of wavelengths by genetic algorithm was

performed before PLS regression in cow and goat milk (Ferrand, 2010). The genetic algorithm used is the algorithm developed by Leardi (1998) which is specific to wavelengths selection. Mutation rate, initial population, and number of variables selected in the solution of initial population were fixed to 1%, 30 and 5 respectively.

GA 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 $\sqrt{\frac{\left(\sum_{i=1}^{N}(\hat{y}_i - y_i)^2\right)}{N-k-1}}$

with N 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 advise to 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.

2. Results and discussion

The calibrations were validated through the accuracy values obtained by validation on a new dataset in cow and ewe milk and by cross-validation in goat milk (Table 1 to 3). About 10 to 20 fatty acids (depending on the species) of 60 have a relative error below 10%. The estimations are better for the FA present in medium or high concentration, i.e. for the saturated fatty acid (C4:0 to C16:0) and for some monounsaturated fatty acids (cis or trans isomers of C18:1). It is worth noting that in the three species, the relative error for the stearic fatty acid (C18:0) is important

The results are comparable in ewe and cow milk. The estimation of lauric acid (c12) is however better in ewe milk. The accuracy is lower in goat milk. This is certainly linked to the lower level of fat in goat milk. But even for the caprylique (C8:0), capric (C10:0), and lauric fatty (C12:0) acids, whose the contents are more important than in ewe and cow milk, the relative error is important (R.E. >12%). New samples in goat milk are intended in the next weeks to improve the accuracy.

Table 1. Statistical parameters for cow milk validation set (PLS regression only or genetic algorithm (GA) + PLS regression)

	N	Mean	Sd	Relative error (%)	R^2
Fat content	70	3.816	0.637	0.32	1.00
C4:0	72	0.149	0.025	5.71	0.88
C6:0	70	0.087	0.015	3.97	0.95
C8:0	70	0.050	0.010	5.00	0.94
C10:0	71	0.111	0.029	6.92	0.93
C12:0	71	0.126	0.037	11.12	0.86
C14:0	72	0.435	0.088	6.10	0.91
C16:0	71	1.271	0.282	6.41	0.92
C18:0	71	0.342	0.099	12.58	0.81
Total 18:1	69	0.780	0.203	6.70	0.93
Saturated	72	2.766	0.510	2.09	0.99
Monounsaturated	69	0.889	0.220	5.80	0.95
Polyunsaturated	69	0.107	0.019	8.06	0.80
Omega 3	70	0.029	0.010	16.24	0.77
Omega 6	70	0.075	0.016	11.23	0.72

Table 2. Statistical parameters for ewe milk validation set (PLS regression)

	N	Mean	Sd	Relative error (%)	R^2
Fat content	54	6.802	1.398	0.40	1.00
C4:0	52	0.233	0.035	5.88	0.85
C6:0	54	0.177	0.033	4.21	0.95
C8:0	54	0.175	0.037	4.83	0.95
C10:0	54	0.574	0.147	5.90	0.95
C12:0	54	0.339	0.103	8.57	0.92
C14:0	54	0.821	0.214	6.98	0.93
C16:0	54	1.650	0.345	6.70	0.90
C18:0	55	0.511	0.143	12.62	0.80
Total 18:1	54	1.276	0.414	4.40	0.98
Saturated	54	4.825	0.994	2.31	0.99
Monounsaturated	54	1.389	0.443	3.83	0.99
Polyunsaturated	55	0.238	0.075	7.03	0.95
Omega 3	52	0.069	0.016	13.65	0.66
Omega 6	55	0.137	0.036	12.13	0.79

Table 3. Statistical parameters for goat milk, cross-validation results (PLS regression only or genetic algorithm (GA) + PLS regression)

	N	Mean	Sd	Relative error (%)	R^2
Fat content	150	3.310	0.666	0.48	1.00
C4:0	150	0.092	0.025	9.23	0.87
C6:0	150	0.078	0.020	8.97	0.87
C8:0	150	0.080	0.022	12.36	0.78
C10:0	150	0.264	0.071	12.48	0.77
C12:0	150	0.134	0.041	13.36	0.79
C14:0	150	0.307	0.077	9.17	0.85
C16:0	150	0.996	0.197	5.14	0.93
C18:0	150	0.282	0.099	18.14	0.73
Total 18:1	150	0.756	0.176	8.84	0.85
Saturated	150	2.351	0.485	3.55	0.97
Monounsaturated	150	0.798	0.184	8.92	0.84
Polyunsaturated	150	0.128	0.028	12.47	0.65
Omega 3	150	0.018	0.005	19.58	0.44
Omega 6	150	0.109	0.027	13.71	0.65

3. Conclusion

These first results show it is possible to obtain accurate estimations for the main fatty acids in individual milk samples of the three species. It was observed that performing a selection of variables prior to the PLS regression permitted to improve accuracy and stabilize equations over the time.

Future researches will focus on other spectrum data pre-treatment procedures, while increasing simultaneously the initial sampling size to get more accurate estimation equations of milk fatty profile.

The advancements of the *PhenoFinLait* program are available on http://www.phenofinlait.fr/.

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Outline

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Material and methods

Results

Conclusions and perspective

Context

- Consumers are aware of the food impact on their health, especially FA
- In France, more and more farmers are paid on the FA composition of their milk

But…

⇒ No reference method to routinely analyze milk FA composition

⇒ No tool (animal genetic and feeding strategy) to adapt fine milk composition to consumers demand

Phénofinlait: How ?

Partners from every rings of dairy industry

CNIEL (Dairy industries and farmers)

France Génétique Elevage
(France Livestock Genetics)

UNCEIA, ANIO and CapGène
(about 10 breeding companies)

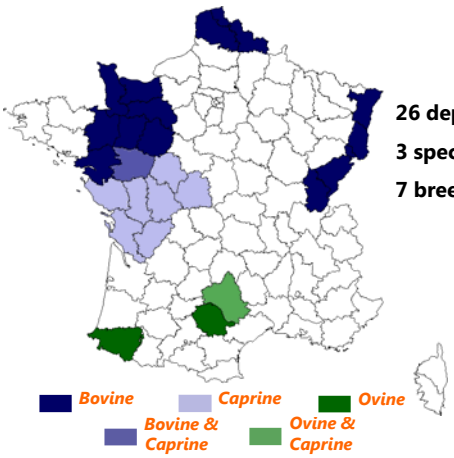
FCL and CNBL
(Milk recording organizations)

Actilait and regional laboratories

INRA
(French National Institute for Agricultural Research)
(4 labo, 4 exp. units, 2 dép.)

Institut de l'Elevage
(French Livestock Institute)
(4 teams, 3 dép.)

And about 1500 farms !



26 departments

3 species,

7 breeds

With the collaboration of Foss and Bentley Instrument / Milk analyzer

PhénoFinLait: aims

- **Develop and control methods to analyze fine milk composition**
- High scale phenotyping and genotyping → 20 000 animals in 1500 farms with fine milk composition, detailed feeding and genotyping
- Understand how genetic and feeding strategies impact fine milk composition
- Create tools (genetics + feeding strategies) to face evolving consumer demands including health requirements

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PhénoFinLait: aims

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How to measure the fine composition in routine?

- MIR spectra routinely obtained by milk recording laboratories for fat and protein percentage measurements
- Possibility to estimate several fatty acids, lactoferrine and some minerals in cow milk

(Université de Gembloux - Soyeurt et al.)

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How to estimate fine milk composition from spectra ?

- Analytical technique based on radiation absorption by molecules
- Linked to the chemical composition : bonds between atoms absorbs infrared energy at characteristic frequencies.
  - ➢ **MIR-Spectrum reflect the biochemical composition**

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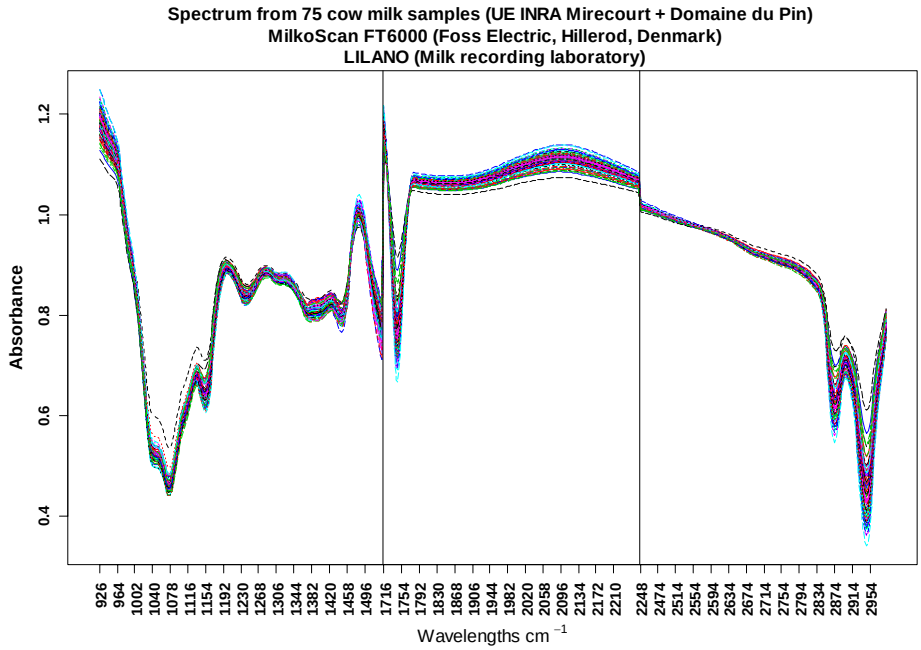

Retained wavelengths :
964-1543 cm^{-1}, 1716-2273 cm^{-1}, 2435-2971 cm^{-1} (Manuel technique Foss)

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How to estimate fine milk composition from spectra ?

- Problem : peaks don't match to fatty acids but to bonds
- Use of PLS regression : allow to take into account the collinearity in the spectrum and to extract the information

➢ **Coefficients are applied to wavelentghs to estimate the fatty acids**

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How to improve equations accuracy ?

- 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 R. 1998, Gomez-Carracedo 2007)
- Previous study in cow milk with good results (Ferrand, 2009)

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Samples analyzed by CPG and MIR

- **Cow:** Pin-Au-Haras, Mirecourt and Méjusseaume experimental farms
  Crossed Holstein X Normande (F2) (150+100), Prim'Holstein and Montbéliard (100), Holstein (96)
- **Goat:** Domaine de Galles farm for Alpine goat (150) + 1 private flock for Saanen goat(50 à 80)
- **Ewe:** Domaine de La Fage for Lacaune ewe (150) + 3 private flock for Manech red faced (30) and Basco-Bearnaise (20)

→ **Almost 75 FA and ratio for each species**

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Results in cow milk

Validation dataset Mirecourt+le Pin	N	Mean	Sd	Relative error (%)	R^2
Fat content	70	3,816	0,637	0,32	1,00
C4:0	72	0,149	0,025	5,71	0,88
C6:0	70	0,087	0,015	3,97	0,95
C8:0	70	0,050	0,010	5,00	0,94
C10:0	71	0,111	0,029	6,92	0,93
C12:0	71	0,126	0,037	11,12	0,86
C14:0	72	0,435	0,088	6,10	0,91
C16:0	71	1,271	0,282	6,41	0,92
C18:0	71	0,342	0,099	12,58	0,81
Total 18:1	69	0,780	0,203	6,70	0,93
Saturated	72	2,766	0,510	2,09	0,99
Monounsat.	69	0,889	0,220	5,80	0,95
Polyunsat.	69	0,107	0,019	8,06	0,80
Omega 3	70	0,029	0,010	16,24	0,77
Omega 6	70	0,075	0,016	11,23	0,72

Estimations of ratios with techno functional or nutritional interests

Validation dataset Mirecourt+le Pin	N	Mean	Sd	Relative error (%)	R^2	Remarks on residuals
index 14	72	7,05	1,461	19,66	0,11	bad fitting
index 16	70	4,31	1,013	12,43	0,72	
index 18	72	67,0	4,100	4,72	0,41	bad fitting
LA/ALA	73	2,79	1,804	18,71	0,92	2 blocks of data; error more important when the ratio is high
OMEGA 6 / OMEGA 3	73	2,92	1,522	19,28	0,86	2 blocks of data; error more important when the ratio is high
C18:1/C16:0	71	0,68	0,252	17,28	0,79	
Atherogenicity index	73	3,17	0,986	10,35	0,89	

Results in ewe milk

Validation dataset	N	Mean	Sd	Relative error (%)	R2
Fat content	54	6,802	1,398	0,40	1,00
C4:0	52	0,233	0,035	5,88	0,85
C6:0	54	0,177	0,033	4,21	0,95
C8:0	54	0,175	0,037	4,83	0,95
C10:0	54	0,574	0,147	5,90	0,95
C12:0	54	0,339	0,103	8,57	0,92
C14:0	54	0,821	0,214	6,98	0,93
C16:0	54	1,650	0,345	6,70	0,90
C18:0	55	0,511	0,143	12,62	0,80
Total 18:1	54	1,276	0,414	4,40	0,98
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Omega 3	52	0,069	0,016	13,65	0,66
Omega 6	55	0,137	0,036	12,13	0,79

Results in goat milk

Cross-validation	N	Mean	Sd	Relative error (%)	R^2
Fat content	150	3,310	0,666	0,48	1,00
C4:0	150	0,092	0,025	9,23	0,87
C6:0	150	0,078	0,020	8,97	0,87
C8:0	150	0,080	0,022	12,36	0,78
C10:0	150	0,264	0,071	12,48	0,77
C12:0	150	0,134	0,041	13,36	0,79
C14:0	150	0,307	0,077	9,17	0,85
C16:0	150	0,996	0,197	5,14	0,93
C18:0	150	0,282	0,099	18,14	0,73
Total 18:1	150	0,756	0,176	8,84	0,85
Saturated	150	2,351	0,485	3,55	0,97
Monounsat.	150	0,798	0,184	8,92	0,84
Polyunsat .	150	0,128	0,028	12,47	0,65
Omega 3	150	0,100	0,031	19,15	0,58
Omega 6	150	0,018	0,005	19,58	0,44

Outline

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Conclusions

- Ambitious multispecies program with a lot of stakes
- Importance to produce robust and accurate equations to estimate milk FA content
- Accurate estimations for saturated fatty acids and some monounsaturated
- It needs to improve accuracy for polyunsaturated fatty acids

Conclusions

Contributions of genetic algorithms

- Accuracy gain of 15% on average
- Notable improvement for FA of a crucial interest regarding human nutrition
- Stabilization of the equations over the time

Prospects

- Validation of the equations by an external organism
- A priori, possibility to work with multispecies equations

Projects

- Estimation of C18:1 trans 10/ C18:1 trans 11 ratio
- Estimations of protein content → 20 000 milks analyzed both MIR and HPLC-MS method
- Improvement of equations by wavelet use

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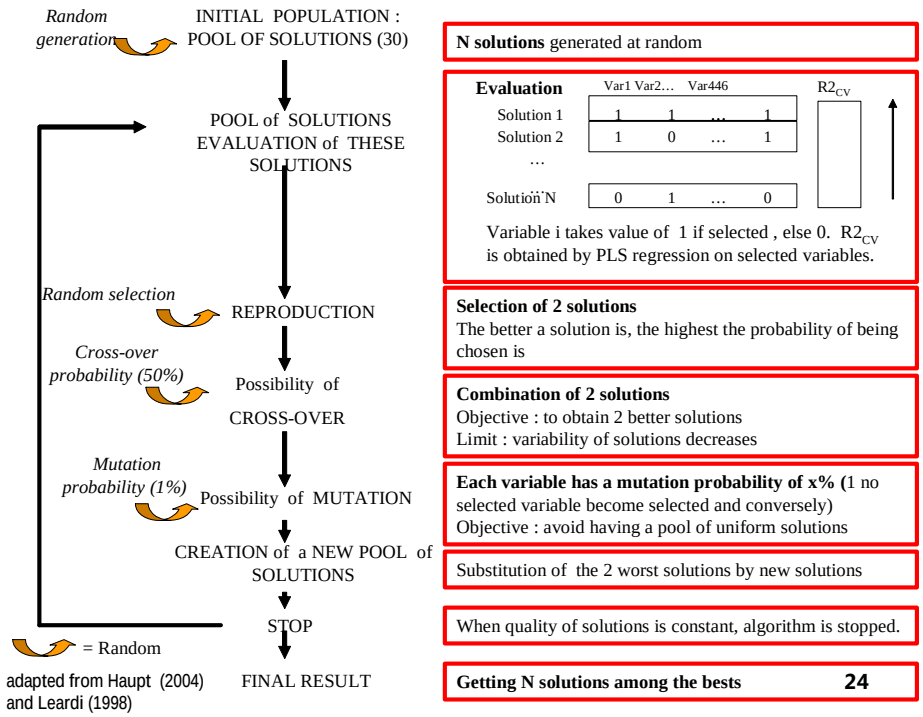
Thank you for your attention !



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New Parameters and Analytical Challenges for Milk Recording by Fourier-Transform Mid-Infrared Spectrometry (FTMIR)

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Abstract

The increasing consumer concern over the relationship between food and human health requires to consider the analysis of new characteristics of milk composition. Due to the large number of analyzed samples, the technology used by the milk recording organizations must be fast and cheap. For these reasons, the Fourier Transform mid-infrared spectrometry (FTMIR) is largely used to quantify major milk components. The recent literature reveals that FTMIR is currently under-used in practice.

Currently, only fat and protein contents are routinely quantified by FTMIR and sometimes the concentrations of urea, lactose, and casein. Recent studies showed the potentiality of FTMIR to quantify new milk components or to predict indicators related to specific milk properties. With the condition of good analytical practices during the calibration and the use of these new equations, some of them can be implemented in milk labs. These FTMIR predictions can be executed internally in the spectrometer software or externally based on recorded spectral data.

Moreover, the FTMIR predictions can be used for additional valorisations by combining information recorded by milk recording structures and the FTMIR predictions. For instance, by using models to explain the observed variability of the studied traits, it is possible to extend the number of possible valorisations such as useful tools for herd management and breeding purposes.

Consequently, FTMIR becomes a powerful technology to quantify milk components and/or to permit a screening of the dairy cattle population based on different milk characteristics interesting for different purposes: nutritional quality (e.g. fatty acid, minerals), hygienic quality (e.g. antibiotics, somatic cells), technological quality (e.g. cheese-making), environment (e.g. methane), herd management (e.g. urea), animal health (e.g. lactoferrin, acetone), and biodiversity. The large number of FTMIR predictions will involve the development of methodologies to resume the most interesting information for the development of specific dairy products and to help farmers in their daily decisions. FTMIR still has a bright future.

Keywords: mid-infrared, milk

1. Introduction

The consumer is more and more conscious that the diversity, the quantity, as well as the quality of the ingested foods influence his health. This situation is reinforced by the attitude of many dieticians and nutritionists who recommend to their patients to limit drastically their consumption of dairy products due to notably the large amount of saturated fatty acids present in bovine milk fat (70% on average). It involves a truncated view in the interest of dairy products. Therefore, to promote the healthiness of dairy products, the dairy sector should take into account the detailed milk composition. Consequently, milk labs and also milk recording organizations should think about the analysis of new characteristics of milk composition showing a potential economic interest.

Traditionally, the assessment of a detailed milk composition is expensive because it requires a lot of different chemical steps and analyses such as the separation of studied constituents from the milk matrix, the use of gas chromatography or other analyses… Moreover, all of these analyses require a lot of time, skilled staff, and use often polluting products. For many years, FTMIR spectrometry has been used to quantify the major components of milk such as fat and protein contents used for the milk

payment. Thanks to its fast and non-destructive advantages, this technology could be a good alternative to the traditional chemical analysis.

2. FTMIR spectrometry

There are 3 different infrared regions (near, medium, and far infrared) with their own specificities. The analysis of milk can be realised by using near or mid-infrared. The mid-infrared has a high sensitivity to the chemical environment due to the fundamental absorptions of molecular vibrations (Belton, 1997). Mid-infrared spectrum represents the absorptions of mid-infrared ray at frequencies correlated to the vibrations of specific chemical bonds (Figure 1). Therefore, the mid-infrared spectrum reflects the global chemical composition. The near infrared gives a much more complex structural information related to the vibration behaviour of combination bonds (Cen and He, 2007). In this review, it was decided to discuss about the potentialities of FTMIR spectrometry for milk analysis because this technology is largely used by milk labs all around the world to quantify major milk components used for the milk payment or by the milk recording organizations to develop management and selection tools to help farmers in their daily decisions.

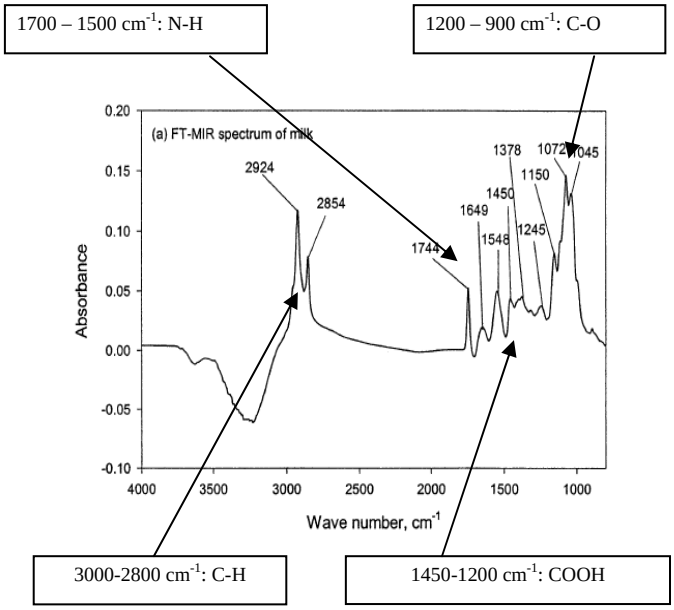

Figure 1. MIR spectrum of milk (Sivakesava and Irudayaraj, 2002)

FTMIR spectrometry is not only routinely used to quantify the contents of fat and protein in milk, but also the contents of urea, lactose, casein, and free fatty acids. Nevertheless, the recent literature reveals that FTMIR is currently under-used in practice.

3. FTMIR and Milk Recording

3.1 Introduction

The main objective of milk recording organizations is to develop management and selection tools useful for the dairy sector including dairy farmers and dairy industry in the current economic context. Two ways are possible to achieve this aim: first, a direct use of the FTMIR predictions of specific milk components and second, the milk recording organization can put together all available information (FTMIR predictions but also animal, lactation, and environmental information) necessary to take into account the natural variation of the considered traits in order to extend the number of potential valorisations.

3.2 Direct Use of FTMIR Data

The principle to obtain milk FTMIR predictions is resumed in Figure 2. The collected samples are analyzed by FTMIR spectrometry and raw data (commonly named spectral data) are generated. The number of datapoints depends on manufacturers. Finally, a specific equation is applied to the spectral data to provide the measurement of the studied trait (e.g., fat, protein…). Therefore, if you want to analyze new components in milk, you need to develop new equations.

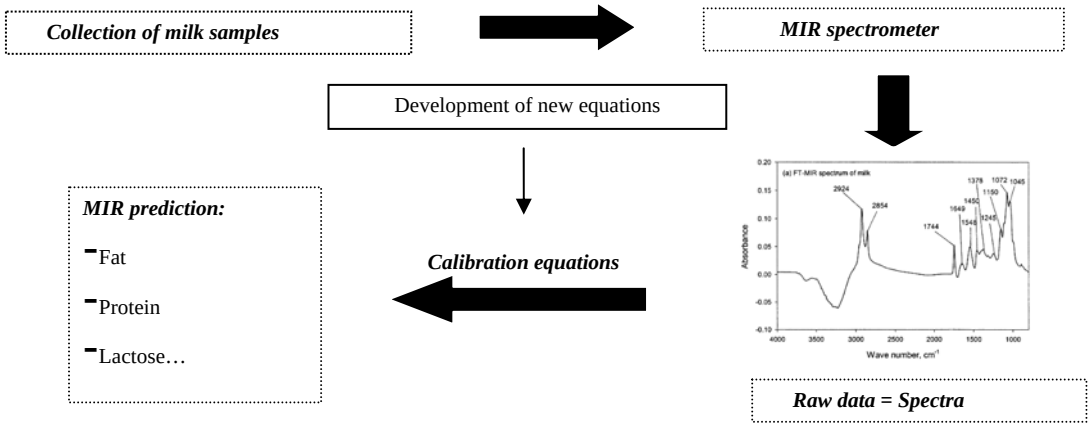

Figure 2. Principle of FTMIR prediction of milk components

Several authors have realized different research studies to extend the number of milk constituents predictable by FTMIR, which showed an interest in different fields such as the nutritional quality (e.g., fatty acid, minerals, lactoferrin), hygienic quality (e.g., antibiotics, somatic cells), technological quality (e.g., cheese-making properties of milk (e.g., casein, titrable acidity, coagulation time…)), environment (e.g. urea, fatty acids, methane emissions through fatty acid predictions (Chilliard et al., 2009)), herd management (e.g., urea, fat, protein, lactose), animal health (e.g., fatty acids, minerals, lactoferrin, β-hydroxybutyrate, acetone), and biodiversity (e.g., by studying the changes in milk composition). This review presents some examples potentially interesting for milk recording organizations.

Recently, several authors showed the potentiality of FTMIR spectrometry to quantify the fatty acid contents directly on bovine milk (Rutten et al., 2009; Soyeurt et al., 2006, 2008a, 2008b, and 2010). The prediction of fatty acid in milk (g/dl of milk) is more accurate if the content of considered fatty acid is high in milk. The FTMIR prediction of fatty acid in fat is less accurate because the variability of fatty acids in milk fat is lower than the one observed in milk. Table 1 describes the results obtained by Soyeurt et al. (2010) from a multiple breeds, multiple countries, and multiple production systems approach for major groups of fatty acids in bovine milk. RPD calculated, as the ratio of the standard deviation of reference value to the standard error of cross-validation, is a parameter assessing the robustness of a calibration equation. If this ratio for a considered equation is greater than 2, it involves a potential use of this equation for breeding and animal purposes. Therefore, all fatty acids shown in Table 1 could be used in practise to assess the nutritional quality of bovine milk fat.

Table 1. Descriptive statistics of the calibration equations for the quantification fatty acids in milk developed by Soyeurt et al. (2010)

Constituent (g/dl of milk)	N	Mean	SD	RPD	SECV
Saturated FA	496	2.40	0.80	15.7	0.0513
Monounsaturated FA	491	1.06	0.37	8.9	0.0411
Polyunsaturated FA	499	0.16	0.05	2.6	0.0204
Unsaturated FA	492	1.22	0.41	9.6	0.0428
Short chain FA	486	0.31	0.11	6.7	0.0165
Medium chain FA	496	1.78	0.60	6.5	0.0928
Long chain FA	495	1.52	0.57	6.5	0.0875

Based on Soyeurt et al. (2009), other traits potentially predictable by FTMIR spectrometry are the calcium, sodium, and phosphorus contents in milk as shown in Table 2. Even if this publication considered a low number of samples, the results for Ca and P were recently confirmed by using 100 additional milk samples (data not shown).

Table 2. Descriptive statistics of the calibration equations measuring minerals in milk developed by Soyeurt et al. (2009)

mg/l de lait	N	Mean	SD	SECV	RPD
Ca	87	1,333	260	95	2.74
K	61	1,336	168	136	1.24
Mg	61	110	18	11	1.68
Na	87	403	107	64	1.68
P	87	1,093	127	50	2.54

Interesting traits for milk recording organizations to check animal health status are ketone bodies. Hansen (1999) and Heuer et al. (2001) developed the first calibration equations to quantify acetone content in bovine milk. More recently, De Ross et al. (2007) has also developed with a relatively good success calibration equations for acetone and β-hydroxybutyrate in milk (Table 3).

Table 3. Descriptive statistics of the calibration equations for ketone bodies in milk developed by De Ross et al. (2007)

mMol	N	Mean	SECV	R^2c
Acetone	1,063	0.146	0.184	0.72
β-hydroxybutyrate	1,069	0.078	0.065	0.62

The improvement of milk nutritional quality is desired. However, it is necessary to know if these changes are positively related to the technological properties of milk. In this context, several authors have developed calibration equations permitting to assess the cheese-making properties of milk through the quantification of specific traits such as titrable acidity, rennet coagulation time… Based on these results (Table 4), it appears that the cheese-making properties of milk could be assessed by relatively good FTMIR predictions of titrable acidity and rennet coagulation time.

Table 4. Descriptive statistics of the calibration equations for traits related to cheese-making properties of milk

		N	Mean	SD	R^2cv	SECV
Titrable acidity (SH°/50ml)	De Marchi et al., 2009	1,063	3.26	0.43	0.66	0.25
Rennet coagulation time (min)	De Marchi et al., 2009	1,049	14.96	3.84	0.62	2.36
	Dal Zotto et al., 2008	74	15.05	3.78	0.73	0.80
pH	De Marchi et al., 2009	1,064	6.69	0.12	0.59	0.07
Titrable acidity (D°)	Colinet et al., 2010	203	16.22	2.01	0.90	0.64
Curd firmness (mm)	Dal Zotto et al., 2008	74	32.43	7.95	0.45	5.49

Another interesting trait is a glycoprotein present naturally in milk and entitled lactoferrin because this molecule is involved in the immune system. Soyeurt et al. in 2007 developed a preliminary calibration equation for the measurement this milk component. This first equation was built with 57 reference samples and the obtained RPD was equal to 2.39 with a SECV equal to 86 mg/l of milk.

3.3 Models based on FTMIR Data

The milk recording organizations have an access to the animal, lactation and environmental data (pedigree, lactation stage, breed, number of lactation…). Merging these data with the FTMIR prediction permits to investigate the potential for using specific models that take into account the natural variability of these FTMIR values and therefore to extend the number of possible valorisations. To illustrate this application, two examples are presented.

Bastin et al. (2009) showed the possibility to model the level of milk urea in a specific herd by using a random regression test-day model. From the results given by the model, it is possible to estimate an expected value of milk urea content in a specific herd at specific test date. Based on that, it is possible to compare the expected value obtained by the model to the observed one. If the difference is too big, it can be assumed that the studied herd has a management problem (Figure 3).

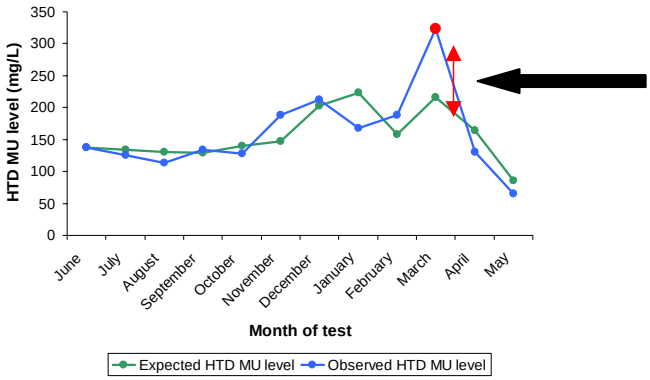

Figure 3. Evolution of observed and expected urea content (MU) in a specific herd (Bastin et al., 2009)

Another very interesting application for milk recording organizations (because these structures have individual values for cows) could be to model the contents of a specific FTMIR prediction in order to give to the farmers sufficient information to discard the less interesting cows and/or to develop an animal selection programs.

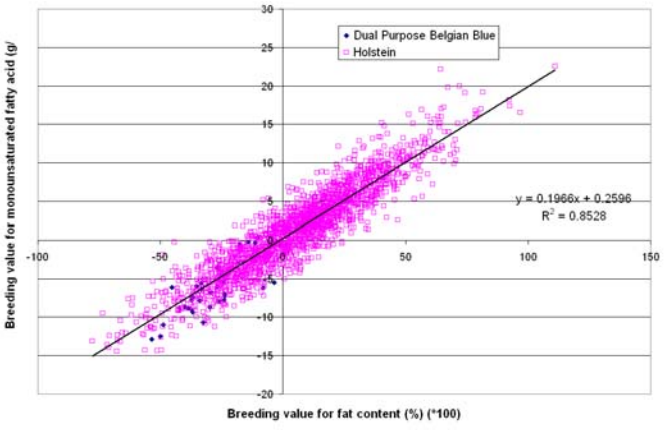

Figure 4. Relationship between the estimated breeding value for monounsaturated fatty acid in milk and the estimated breeding value for fat content

One interest of an animal selection program is to have information for foreign bulls based on data collected from their daughters present in a country where the FTMIR analysis of a specific trait is done. For instance, through the European project RobustMilk (www.robustmilk.eu), the tools needed to implement an animal selection programs for fatty acid contents in bovine milk are developed. The contents of fatty acids in milk are heritable. The lactation heritabilities for saturated and

monounsaturated FA were 44% and 22%, respectively. The first results were obtained from data collected from first parity cows. Figure 4 shows the results of the genetic evaluation for 1,993 bulls with a sufficient number of Walloon daughters with known fatty acid data. High variability of breeding values (parameters estimated to assess the individual variability of studied animals) for monounsaturated fatty acid content was observed for a considered estimated breeding value of fat content (Figure 4). Consequently, a sufficient variability of fatty acid traits exists to investigate the development of an animal selection based on the improvement of the nutritional quality of milk.

This kind of researches can be extended to all FTMIR predictions.

4. Conclusion

In conclusion, the FTMIR spectrometry is currently under-used in practice even if new traits predictable by MIR exist. A lot of work should be done by the milk recording organizations to include these new traits showing a potential economic interest in their services given to their members. Two ways will be possible to achieve this objective: a direct use of FTMIR predictions given by milk labs and/or the developments of specific models taking into account the natural variability of the studied infrared traits in order to develop specific valorisations for dairy sector including farmers, dairy companies, breeding associations…

However, this introduction of new traits in the routine milk recording will involve new challenges. The first challenge will be an analytical challenge. To avoid high bias, the FTMIR equation should be validated on the considered cow population. Indeed, breed differences or differences in the milk samples (e.g., the composition of bulk milk is less variable than the composition of milk samples collected from individual cows) used to develop the calibration equation could involve a bias. Moreover, it is currently possible to implement externally new equations thanks to the recording of spectral data. However, to use this approach successfully, it will be needed that the variability of the spectral data used for the prediction by the milk recording organization was taken into account in the calibration set used to build the used FTMIR equation. Finally, the accuracy of the FTMIR prediction should be tested regularly by the use of reference samples to correct if needed the bias and the slope of the calibration equation. Since January 2008, FTMIR fatty acid predictions is implemented in the Walloon milk lab (Battice, Belgium) and a maintenance is realized using milk samples with known contents of fatty acid (these samples are produced by Walloon Agricultural Research Centre – Valorisation of Agricultural Products Department (Gembloux, Belgium)).

The second challenge will be a computational challenge. The number of studied traits by milk recording organizations will increase. Consequently, it will be necessary to study some traits simultaneously because some of them (the majority of them) will be correlated. It will be also important to know the natural variability of the studied FTMIR trait for a specific cow because the optimum of content for a studied trait can be different according to the considered aim. For instance, high lactoferrin content in milk is interesting for human health but sick cows can also produce milk samples with high content of lactoferrin. This kind of applications will require the use of multiple traits models, which need high computational cost.

In conclusion, a lot of work to do to improve the services given to the dairy farmers thanks to the extension of FTMIR possibilities.

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New Parameters and Analytical Challenges for Milk Recording by Fourier-Transform Mid-Infrared Spectrometry (FTMIR)

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ICAR – 1st June 2010 – Riga – Latvia

Why mid-infrared?

- Advantages of **InfraRed Spectrometry** :
  - Fast
  - No destructive method
  - Environmentally friendly
- Near infrared *vs.* mid-infrared (MIR) :
  - **MIR** : high sensitivity to the chemical environment due to the fundamental absorptions of molecular vibrations (Belton, 1997)
  - **NIR** : much more complex structural information related to the vibration behavior of combination bonds (Cen and He, 2006)

MIR Spectrum

1700 – 1500 cm^{-1} : N-H

1200 – 900 cm-1 : C-O

- ***MIR spectrum*** : absorptions of IR at frequencies correlated to the vibrations of specific chemical bonds within a molecule (Coates, 2000)
- Typical chemical composition (Smith, 1996)

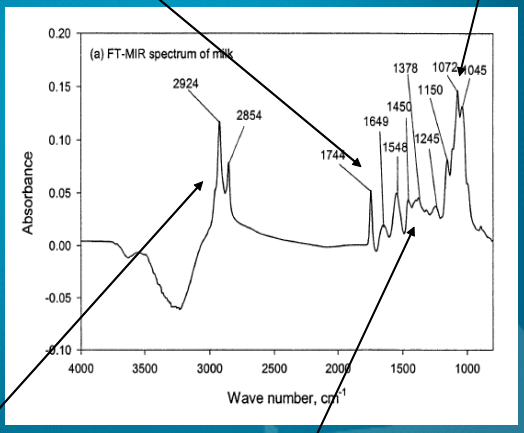

Figure 1 : MIR spectrum of milk (Sivakesava and Irudayaraj, 2002)

3000-2800 cm^{-1}: C-H

1450-1200 cm-1 : COOH

Why mid-infrared?

- Other advantages of **MIR spectrometry**:
  - Largely used by milk labs to quantify the major components of milk
  - Milk samples collected for the milk payment or for the routine milk recording are analyzed by MIR

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General aim

Université de Liège

- **Aim** : Development of management and selection tools useful for the dairy sector including dairy industry and dairy farmers in the current economic context
- ***How ?***
  - Direct use of the results obtained from calibration equations which predict the contents of specific milk components

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Interest

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Animal Health
Fatty acids, minerals, lactoferrin, lactose, β-hydroxybutyrate, acetone…

Environment
Fatty acids, methane emission through fatty acid contents, urea

Herd Management
Urea, fat, protein, lactose…

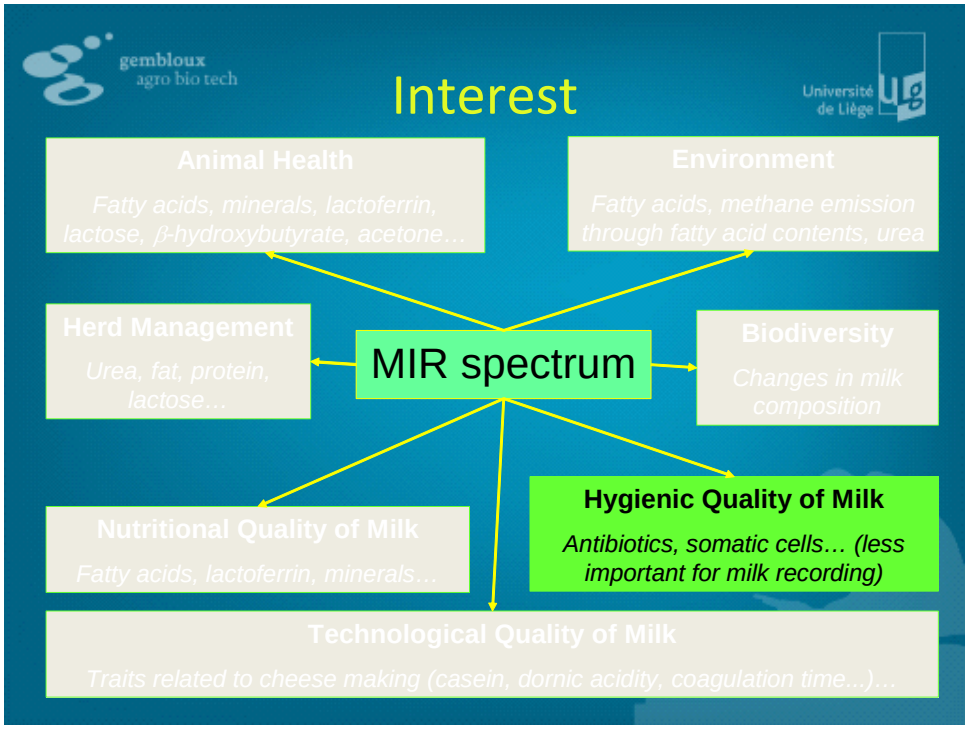
MIR spectrum

Biodiversity
Changes in milk composition

Nutritional Quality of Milk
Fatty acids, lactoferrin, minerals…

Hygienic Quality of Milk
Antibiotics, somatic cells… (less important for milk recording)

Technological Quality of Milk
Traits related to cheese making (casein, dornic acidity, coagulation time...)…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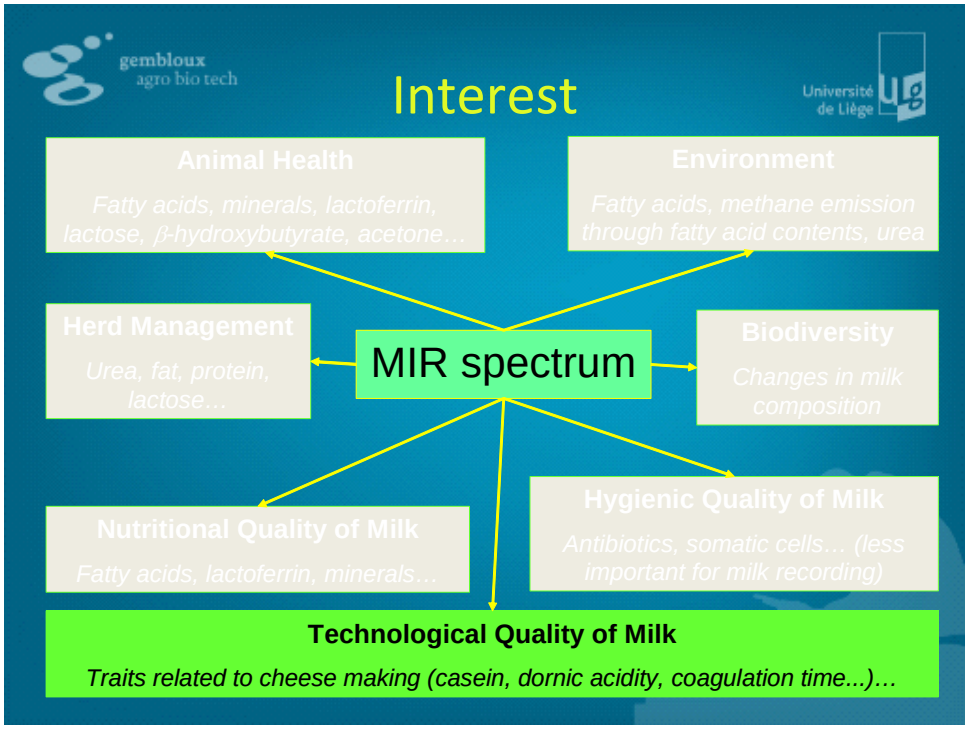
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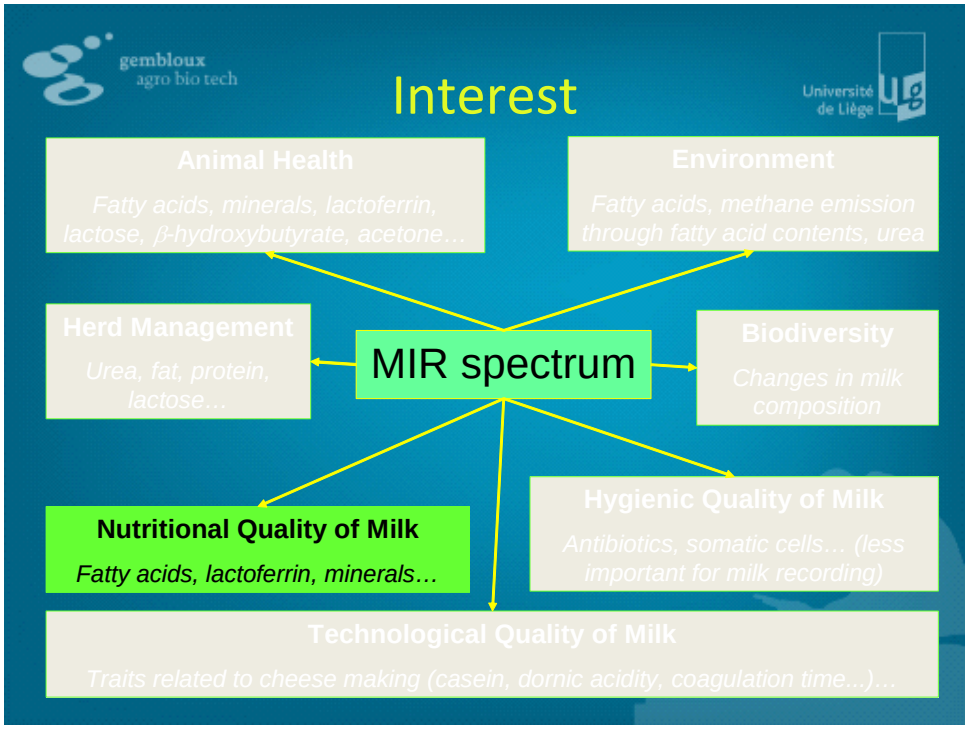
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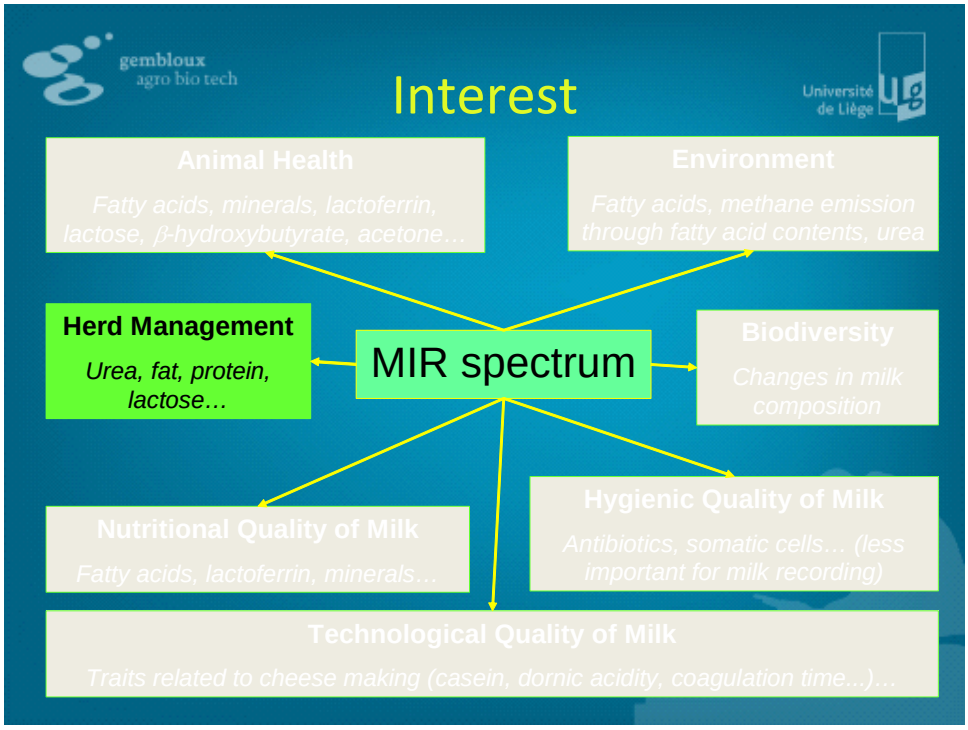
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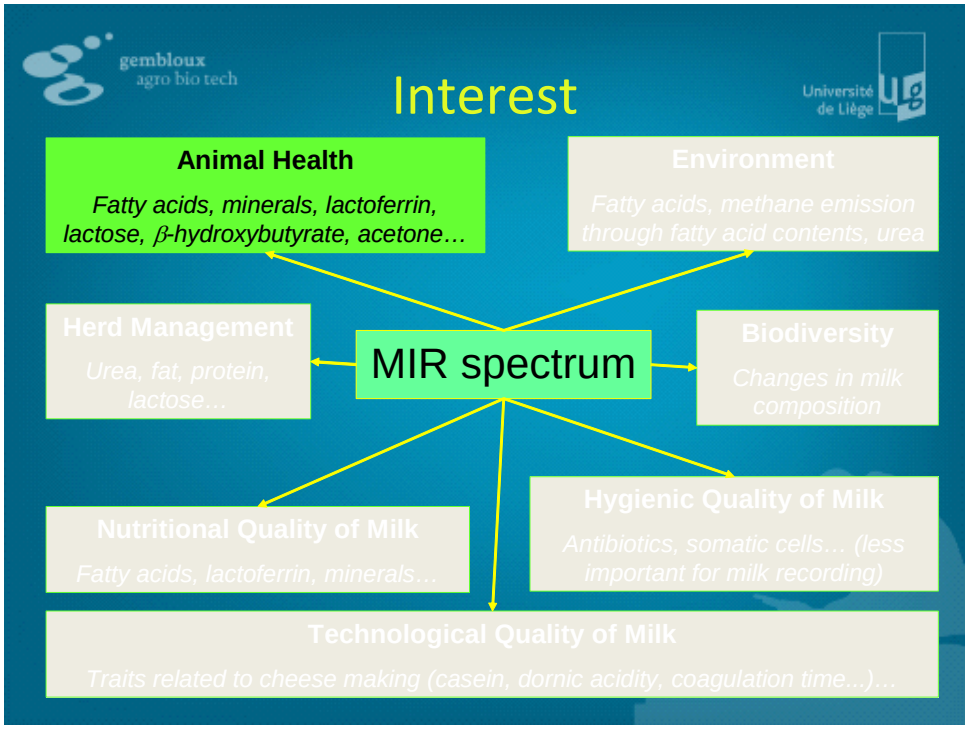
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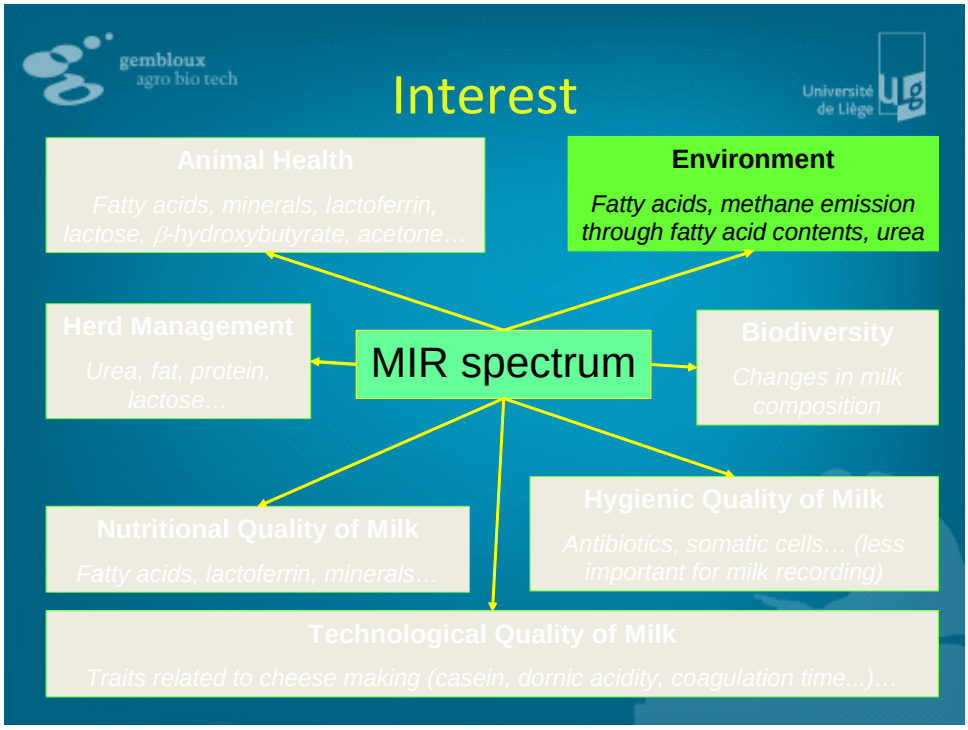
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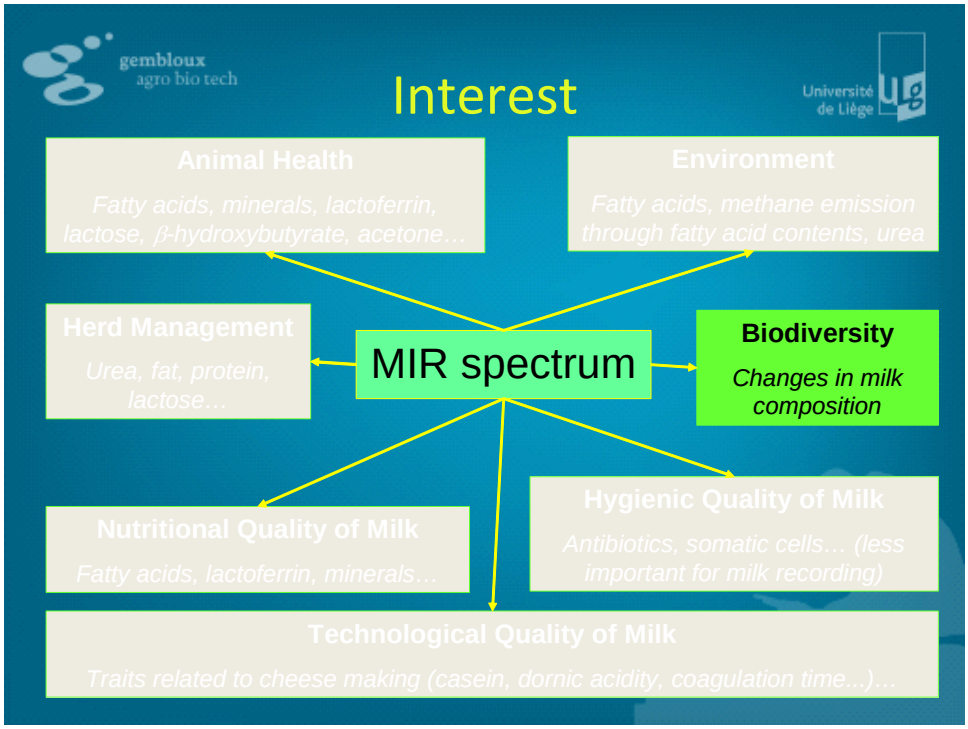
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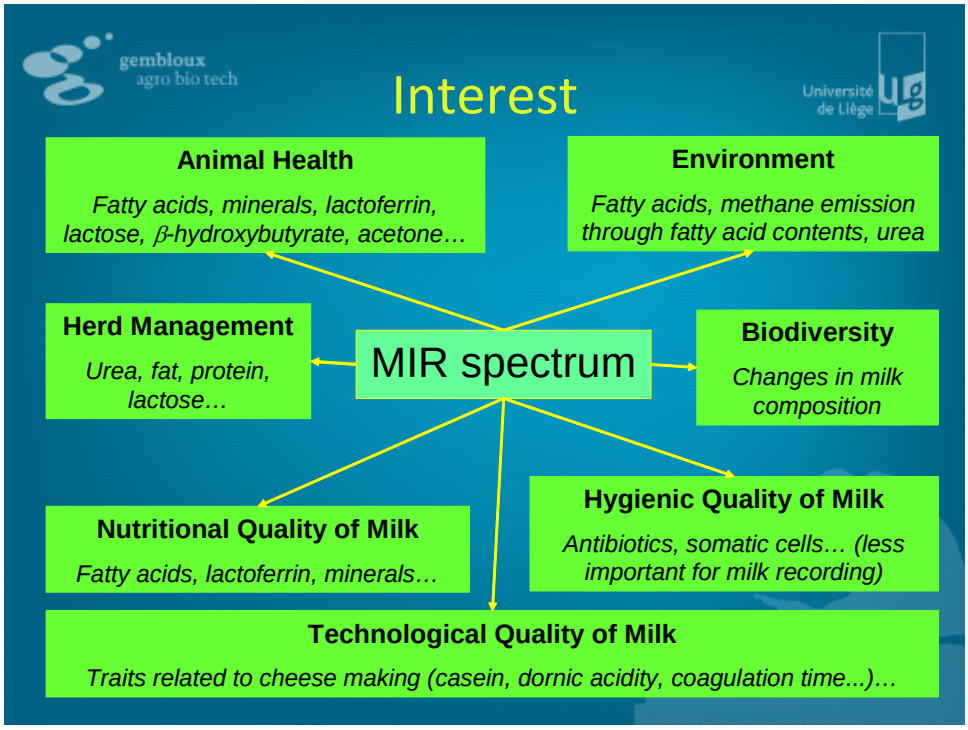
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Interest
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Animal Health
Fatty acids, minerals, lactoferrin, lactose, β-hydroxybutyrate, acetone…
Environment
Fatty acids, methane emission through fatty acid contents, urea
Herd Management
Urea, fat, protein, lactose…
MIR spectrum
Biodiversity
Changes in milk composition
Nutritional Quality of Milk
Fatty acids, lactoferrin, minerals…
Hygienic Quality of Milk
Antibiotics, somatic cells… (less important for milk recording)
Technological Quality of Milk
Traits related to cheese making (casein, dornic acidity, coagulation time...)…

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Common traits

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- Fat content
- Protein content
- Urea
- Lactose
- Casein
- Free fatty acids

Milk payment + milk recording

Milk recording

Few milk recording

➔ Recent studies showed that the MIR spectrometry is currently under-used

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Principle

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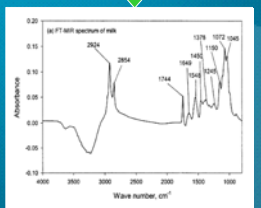
Collection of milk samples

(Foss, 2008)

MIR spectrometer

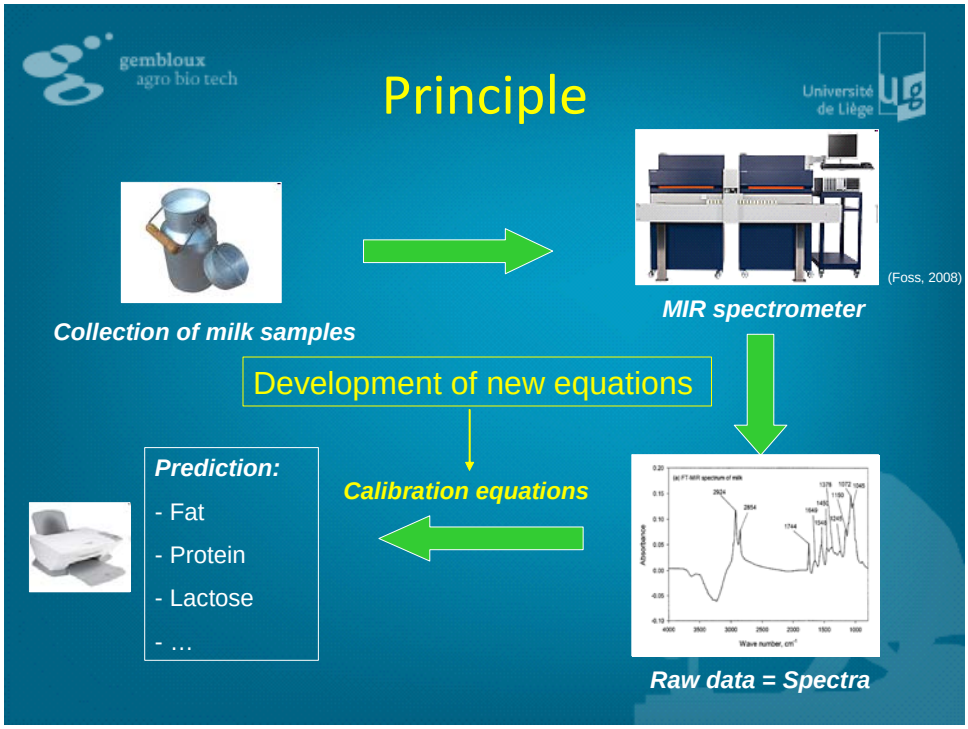


Raw data = Spectra

Calibration equations

Prediction:

- Fat
- Protein
- Lactose
- …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Principle
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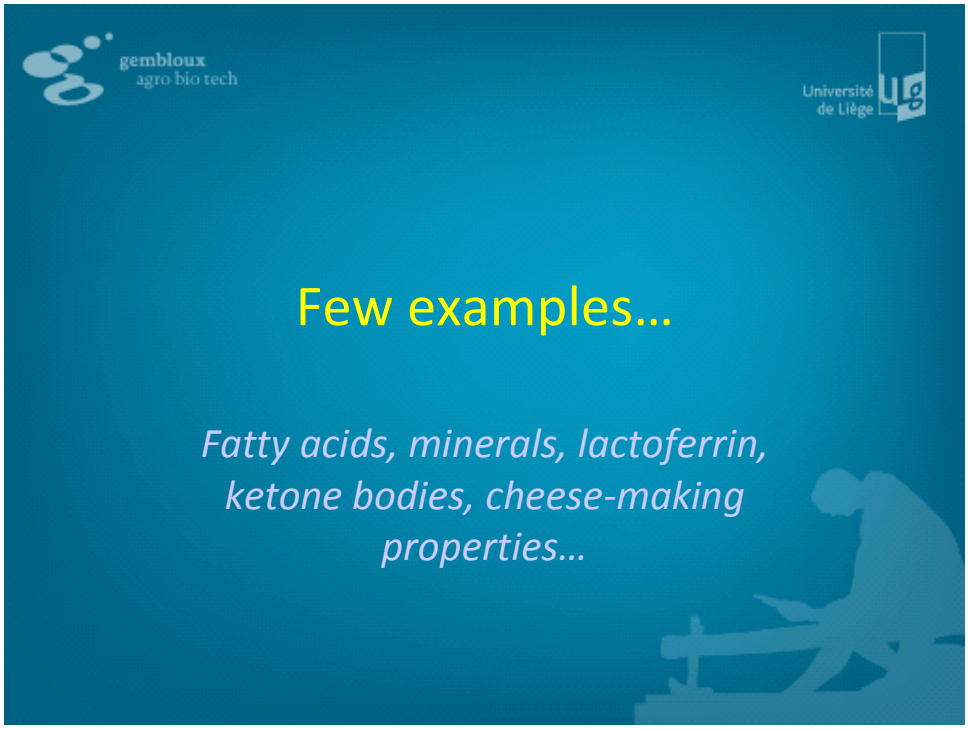
(Foss, 2008)
MIR spectrometer
Collection of milk samples
Development of new equations
Prediction:
- Fat
- Protein
- Lactose
- …
Calibration equations
Raw data = Spectra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Few examples…
Fatty acids, minerals, lactoferrin, ketone bodies, cheese-making properties…

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Fatty Acids (FA)

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- Recent studies confirmed the ability of MIR to predict FA in milk (g/dl of milk):
  - Soyeurt et al. (2006, 2008, 2009), Rutten et al. (2009)
- Lower ability to predict FA content in fat (g/100g of fat)
- New results obtained in the RobustMilk project (www.robustmilk.eu)
  - Multi-breeds, multiple countries and multiple production systems
- All studied FA have a RPD (SD/SECV) greather than 2

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Fatty Acids

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Constituent (g/dl of milk)	N	Mean	SD	RPD	SECV
Saturated FA	496	2.40	0.80	15.7	0.0513
Monounsaturated FA	491	1.06	0.37	8.9	0.0411
Polyunsaturated FA	499	0.16	0.05	2.6	0.0204
Unsaturated FA	492	1.22	0.41	9.6	0.0428
Short chain FA	486	0.31	0.11	6.7	0.0165
Medium chain FA	496	1.78	0.60	6.5	0.0928
Long chain FA	495	1.52	0.57	6.5	0.0875

H. Soyeurt, F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne. 2010. J. Dairy. Sci. Submitted.

This study will be presented in details at ADSA conference in July at Denver (USA)

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Minerals

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- First results were published by Soyeurt et al., 2009

mg/l de lait	N	Mean	SD	SECV	RPD
Ca	87	1,333	260	95	2.74
K	61	1,336	168	136	1.24
Mg	61	110	18	11	1.68
Na	87	403	107	64	1.68
P	87	1,093	127	50	2.54

- Current study confirmed these results with a larger database (more than 100 samples)

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Lactoferrin

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mg/l de lait	N	Mean	SD	SECV	RPD
Lactoferrin	57	253	206	86	2,39

- Milk glycoprotein involved in the immume system defenses
- Preliminary results published in 2007
- Validation in the RobustMilk project (www.robustmilk.eu) on more than 3,000 data

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Ketone Bodies

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- Acetone: Hansen (1999) and Heuer et al. (2001)
- De Roos et al. (2007) studied also 2 other ketone bodies (N spectral data > N sample)

mMol	N	Mean	SECV	R^2c
Acetone	1,063	0.146	0.184	0.72
β-hydroxybutyrate	1,069	0.078	0.065	0.62

De Roos et al., 2007

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Cheese-Making

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		N	Mean	SD	R^2cv	SECV
Titrable acidity (SH°/50ml)	De Marchi et al., 2009	1,063	3.26	0.43	0.66	0.25
Rennet coagulation time (min)	De Marchi et al., 2009	1,049	14.96	3.84	0.62	2.36
	Dal Zotto et al., 2008	74	15.05	3.78	0.73	0.80
pH	De Marchi et al., 2009	1,064	6.69	0.12	0.59	0.07
Titrable acidity (D°)	Colinet et al., 2010(*)	203	16.22	2.01	0.90	0.64
Curd firmness (mm)	Dal Zotto et al., 2008	74	32.43	7.95	0.45	5.49

(*) These results will be presented by Colinet at «New Technologies » session on Friday at 10:50 am

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General aim

- **Aim** : Development of management and selection tools useful for the dairy sector including dairy industry and dairy farmers in the current economic context

- ***How ?***
  - **Direct use** of the results obtained from calibration equations which predict the contents of specific milk components
  - Integration of these infrared predictions in specific models taken into account the variability of these values in order to extend the number of possible valorizations

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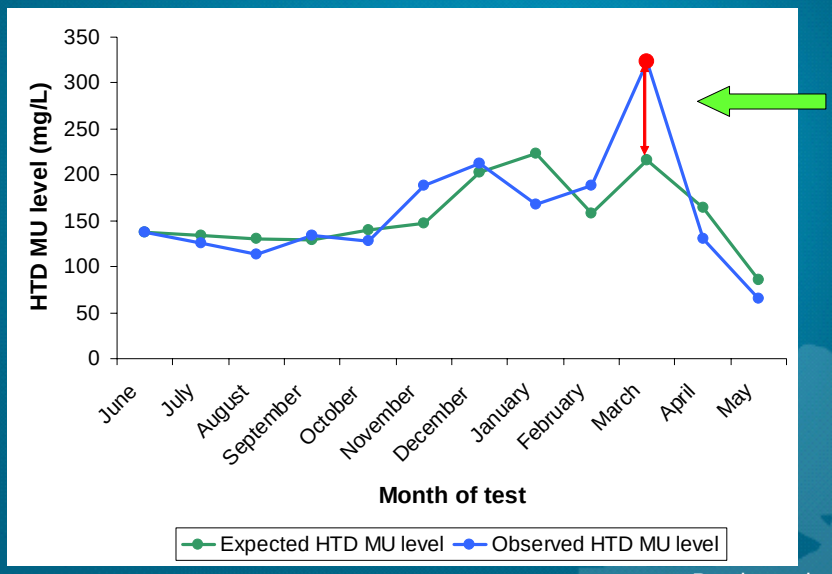
Few examples…

Urea



Bastin et al., 2009

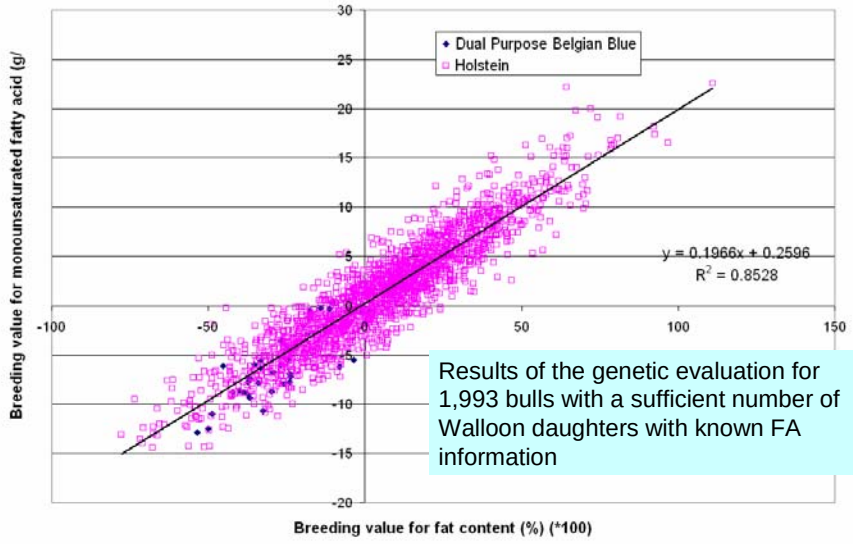
Fatty Acids

- Potential used of FA predictions (*milk labs*):
  - FA predicted from bulk tank milk:
    - Separate scheme of milk collecting
    - Subsidy given in Belgium by a dairy company for milk with higher unsaturated FA
  - FA predicted from individual cows (*Milk recording*)
    - To discard cows
    - Animal selection programs
      - Most interesting bulls and dams
      - Possible internationalization based on relationships among animals

Fatty Acids

- Animal selection program for FA
  - RobustMilk project (www.robustmilk.eu)
  - Heritable trait with sufficient genetic variability
    - Saturated FA: +/- 44% (more than fat content)
    - Monounsaturated FA: +/- 22%
  - Feasability of genetic selection
    - Genetic evaluation for cows in first lactation
    - Results will be presented at INTERBULL session (Nicolas Gengler on Wednesday at 8:00 am) and at ADSA conference in Denver

Fatty Acids



Results of the genetic evaluation for 1,993 bulls with a sufficient number of Walloon daughters with known FA information

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Fatty Acids

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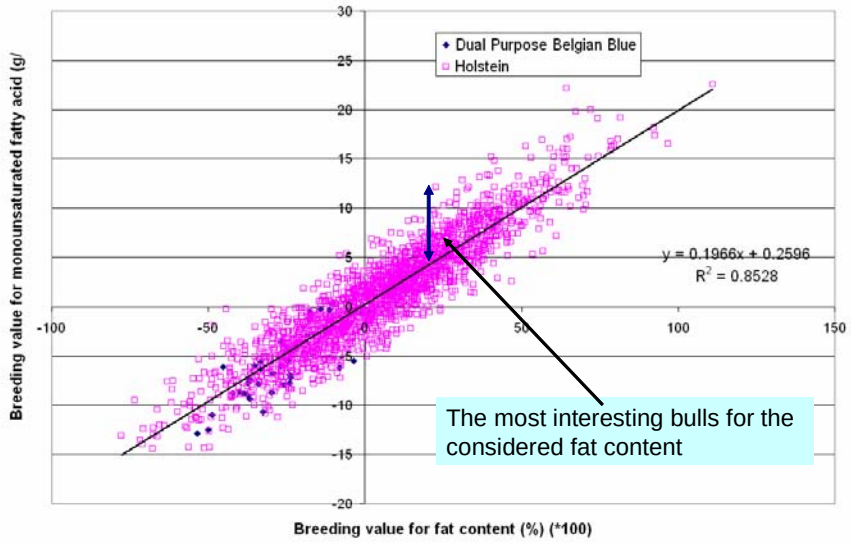

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Conclusions

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- MIR is currently under used in practice
- New parameters predictable by MIR exist with potential interests for milk recording:
  - Directly MIR predictions
  - Models to offer specific valorizations for dairy industry (farmers, dairy companies, breeding associations…)

But … (analytical challenges)

- The MIR equation used should be validated on the considered cow population:
  - Breed differences can appear
  - Milk from bulk tank is less variable than milk samples collected from individual cows
- Currently, it is possible to implement externally the new equations thanks to the recording of spectra
  - Make sure that the variability of the spectral data used for the prediction was taken into account in the calibration set used to build the calibration equation
- The accuracy of the MIR prediction should be tested regularly by the use of reference samples
  - Since January 2008, MIR FA predictions is implemented in the Walloon milk lab → a maintenance is realized using milk samples with known contents of FA

But … (computational challenges)

- The number of studied traits will increase
  - Some traits are correlated → for the development of specific valorizations for breeders, it will be important to know the relationships among studied traits
    - e.g., fatty acids vs. protein, …
  - The optimum of content for the studied trait can be different following the considered aim
    - e.g., high lactoferrin in milk interesting for human health vs. Milk sample with high content of lactoferrin can be produced by a sick cow → take into account the natural variation of each studied trait

→ multiple traits models → high computational cost

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A lot of work to do …

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Collaborators for our researches

- **GxABT :**
  - Nicolas Gengler - Valérie Arnould – Catherine Bastin - Alain Gillon - Sylvie Vanderick
- **CRA-W :**
  - Frédéric Dehareng - Pierre Dardenne
- **Comité du Lait :**
  - Didier Veselko – Emile Piraux
- **AWE :**
  - Carlo Bertozzi – Laurent Laloux – Xavier Massart

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Thank you for your attention

MIR spectrometer

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Herd Navigator - Real Time Herd Management, or "How to benefit from frequent measurements!"

Tove Asmussen

FOSS A/S, Hillerød , Denmark

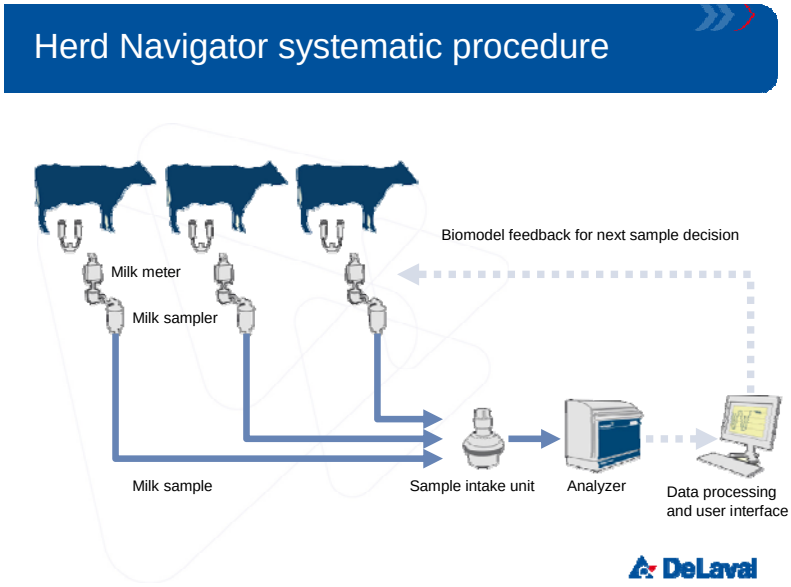
Abstract

With Herd Navigator frequent measurements ensures close monitoring of the herd to allow pro active action on a number of parameters such as reproduction parameters, mastitis, ketosis and urea.
It is evident that alerts informing the herd manager about need for insemination or other immediate actions are of big value, - however it is also important to take advantage of the frequents measurements for more proactive activities on group or herd level.
This presentation will give examples on such possibilities.

Keywords: herd monitoring, milk, analysis, health, reproduction, mastitis, ketosis

1. Introduction to Herd Navigator

The solution works by taking a representative milk sample of individual cows during milking. The milk is taken at the milk samplers connected at each individual milking point or milking robot.



When the cow is being milked the sampler delivers its milk to the sample intake unit (SI) located at the end of the milking pit. This device holds the samples and sends them one by one to the Analyzer Instrument (AI) located in the milk room.

The Analyser Unit is temperature and humidity constant and uses dry stick technology to perform the analysis. Each parameter has its own stick and those are stored in cartridges inside the AI. The parameters measured are:

Focus area	Parameter analyzed in milk	Early/on time detection
Reproduction	Progesterone	Heat Silent heat Pregnancy Abortion Cysts Anoestrus
Udder health	LDH – lactate dehydrogenase	Mastitis Subclinical mastitis
Feeding and energy balance	Urea BHB – beta hydroxybutyrate	Feed ration – protein Ketosis Subclinical ketosis Secondary metabolic disorders

The technique used for LDH, urea and BHB is colorimetric and for progesterone it is lateral flow assay.

The values are captured by the biological model which calculates the risk of any of the above diseases or physiological statuses and at the same time decides when each parameter will be measured again next time for the cow in question.

The complete system gets cleaned automatically together with the milking machine equipment.

2. How to maximize the benefit of frequent measurements

Herd Navigator impacts on the most important factors on milk production, reproduction, mastitis and feeding. All information from Herd Navigator can, one way or the other, be combined with information already present in the on farm cattle database and the central cattle database.

Herd Navigator detects consistently above 95% of all heats of the herd (including silent ones) and is able to pin point the time of the heat, the likelihood of success of a prospective insemination as well as the system is able to detect post partum anoestrus, pregnancy and both types of ovarian cysts. This has resulted in a significant reduction on open days at most farms running a Herd Navigator.

Herd Navigator is able to detect clinical and sub-clinical mastitis up to 3 to 4 days before clinical signs are shown in the animals affected. The sensitivity of the system reaches more than 80%.

Herd Navigator is able to detect all cases of clinical and subclinical ketosis, and normally it detects 50% more ketotic cows than do the farmers/herd manager.

For an average European herd the data shows that Herd Navigator can bring profit improvement potentials for farmers from 250 to 350 € per cow per year.

Benefiting to this extent from running Herd Navigator requires optimum use of results from the Herd Navigator. Herd Navigator both provides new information and more frequent information than most dairy farmers have been used to previously. All this information is combined with the already existing information in the farms herd management system, - but to benefit further from all the acquired information a more advanced tool is under development.

In the following a few examples will be presented.

2.1 Ketosis

The level of BHB in milk is monitored from calving until 60 days after calving.

The incidence of ketosis varies significantly from herd to herd and from one period to another.

In general we have seen that the frequency is significantly higher than registered by the herd manager before Herd Navigator is introduced. Though all present Herd Navigator users have doubts about the correctness of the alerts issue there is big differences in the way they act upon the alerts and thereby on the effect on performance in the herd.

By monitoring the lactation cumulated lactation curve of all cows having a ketosis alert in different herds it is clearly seen that the yield loss in some herds are almost avoided whereas it is very big over the entire lactation in other herds.

2.2 Reproduction

The progesterone level is monitored frequently from 15 days after calving until 60 days after the last heat. At this stage the cow is considered pregnant, and the risk of abortion limited. This allows monitoring for prolonged anoestrus, heat, follicular and luteal cysts, pregnancy, early foetus loss (day 24-35) and abortion >day 35).

On average this has allowed the HN test farms to reduce no of empty days by 22 days, - though reduction in number of empty days has not been main targets in all herds. However an additional benefit of the Herd Navigator, besides informing about when to inseminate which cow, is to display why cows are not getting pregnant.

Is it because inseminations are badly timed? Do cows become pregnant, - but suffer from early foetus loss? Or do they abort more than 35 days after insemination. These questions can be answered by examining the progesterone curves of the cows.

Doing so in different test herds has revealed big differences in the reasons behind reproduction problems. Knowing the reason for a problem can eventually lead to solving the problem by changes in management procedures, feeding or other measures.

3. Conclusion

Above two examples illustrates that frequent analyses of management parameters give access to further information about the reasons behind potential problems in a herd.

It is important to reveal such information to the herd manager and the information may also be important to other parties.

It can therefore be concluded that:

1) It is important to use the new information available to develop new parameters to be monitored in order to optimize the production and economy in the herd.
2) It is important to use the new information available to monitor the breeding goals in new ways.

Herd Navigator - real time herd management

How to benefit from frequent measurements!

Tove Asmussen

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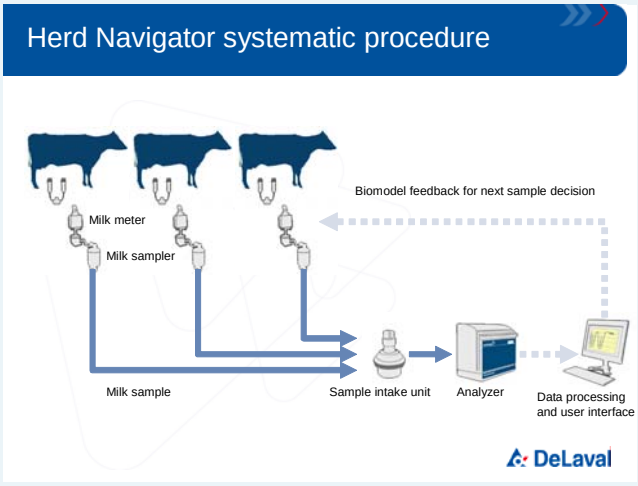
Herd Navigator - an overview:



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Parameters analysed

Focus area	Parameter analyzed in milk	Early/on time detection
Reproduction	Progesterone	Heat Silent heat Pregnancy Abortion Cysts Anoestrus
Udder health	LDH – lactate dehydrogenase	Mastitis Subclinical mastitis
Feeding and energy balance	Urea BHB – beta hydroxybutyrate	Feed ration – protein Ketosis Subclinical ketosis Secondary metabolic disorders

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Moving from monthly towards daily information opens for new parameters to be monitored

- Milk recording offering monthly information
- Bulk samples analysed offers more frequent analyses
- Breeding information, from daily to monthly!
- Milk meters etc in the parlor or analyses equipment connected to Milking Robots gives access to daily information
- Herd Navigator gives daily update on reproduction, mastitis, metabolic disorders and feeding, and recommend actions

4

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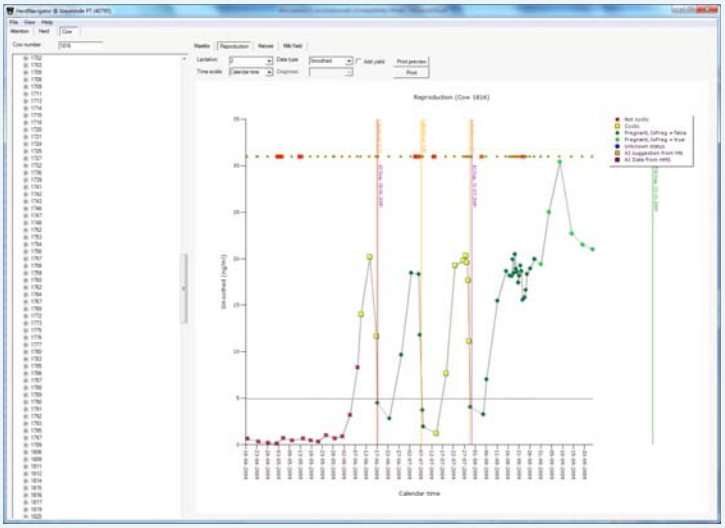
Progesteron curve: Normally cycling cow becomes pregnant!



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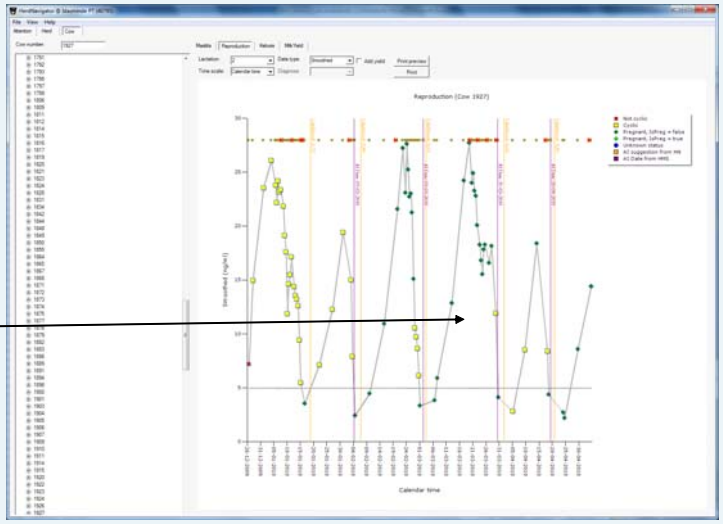
Cycling cow has Early Embryo Death



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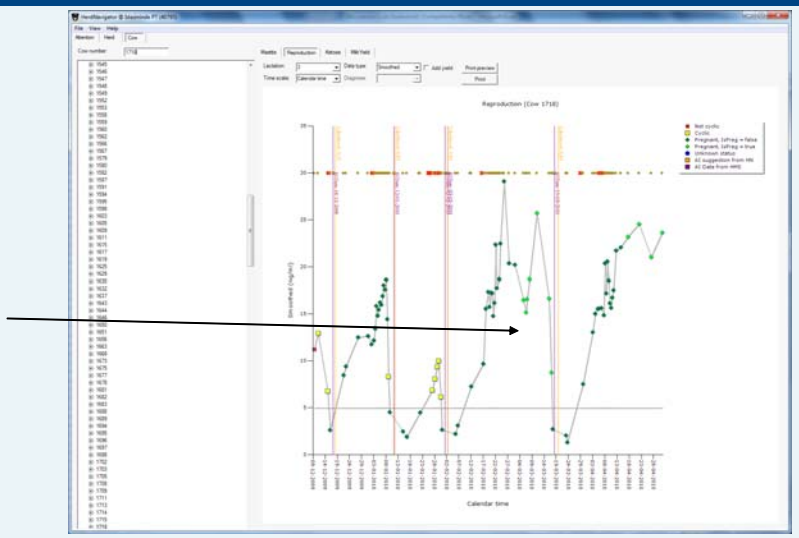
Cow having an abortion 7 weeks pregnant



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Reproduction data, 4 herds

	Farm 1	Farm 2	Farm 3	Farm 4
No of cows started	76	188	198	119
Heat det. Rate %	95.2	96.2	96.5	96.8
No of cows pregnant	66	149	158	92
No of AI, pregn cows	99	316	261	189
% pregnant 1st AI	67	41	56	49

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A closer look:

	Farm 1	Farm 2	Farm 3	Farm 4
No of cows started	76	188	198	119
Heat det. Rate %	95.2	96.2	96.5	96.8
Post Partum Anoestrus (%)	**10.5**	17.6	13.6	**31.9**
Early Embryo Death %	**10.6**	19.6	16.5	18.4
Abortion (>35 days) %	11.8	14.5	12.9	16.3
Follicular Cysts %	22.4	**15.4**	21.2	32.8
Luteal Cysts (%)	21.1	**12.2**	24.7	23.5

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Conclusions Reproduction

At single cow level:

- Non-pregnant cows are detected immediately!
- Pregnancies can be checked without manual pregnancy check
- Reproductive disorders (e.g. cysts) can be detected immediately

At herd level:

- Much more precise reasons for repro problems can be pointed out
  - E.g. irregular cycles in early lactation due to excessive mobilization of body fat (high yielders)

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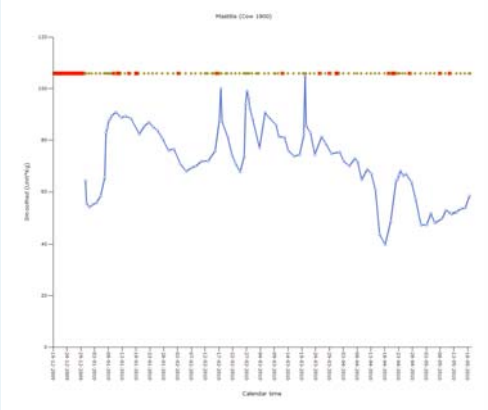
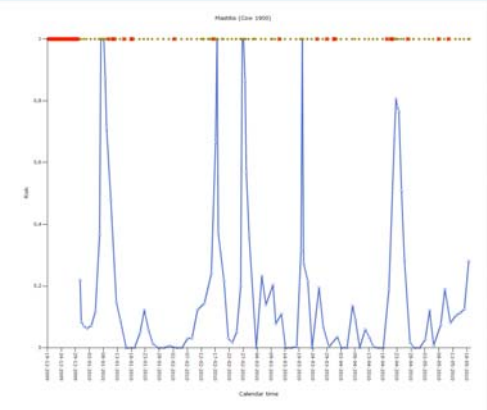
Mastitis alert, 1st time and chronical case





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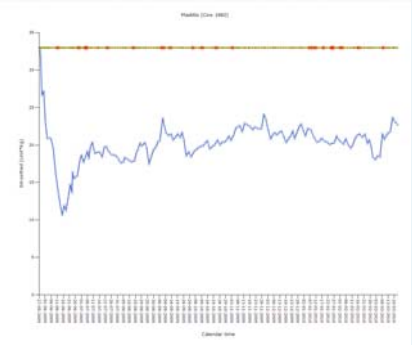
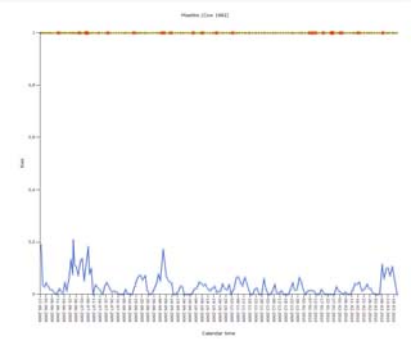
Udder status = Health





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Mastitis alerts, distribution throughout lactation

	Farm 2	
no of cows	37	
alerts, no of	1	2 to 4
Split in alerts, %	54	46
Occur < 25 days, %	27	0
25<x<100 days, %	36	68
later than 100 days, %	36	32

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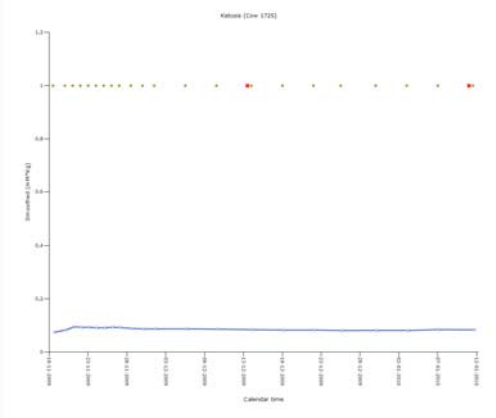
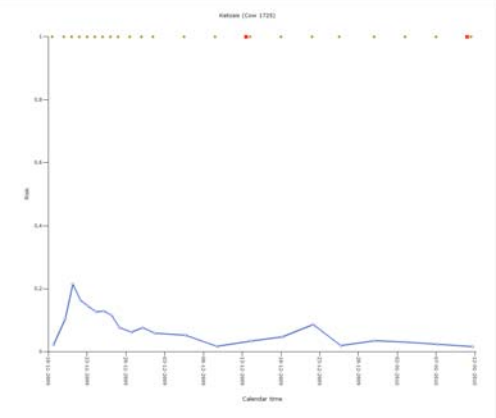
Monitoring ketosis





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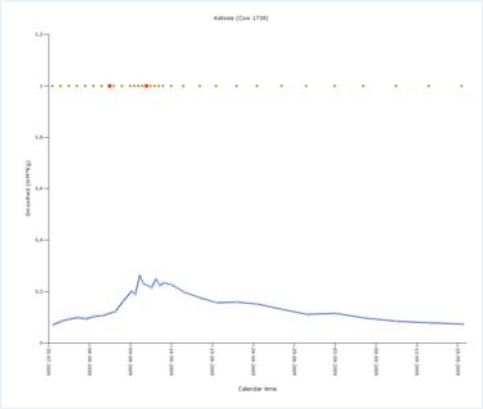
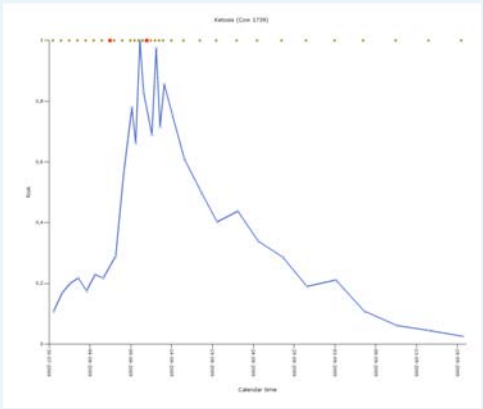
Subclinical ketosis alert





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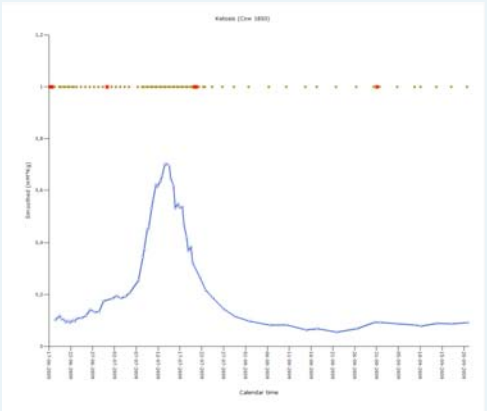
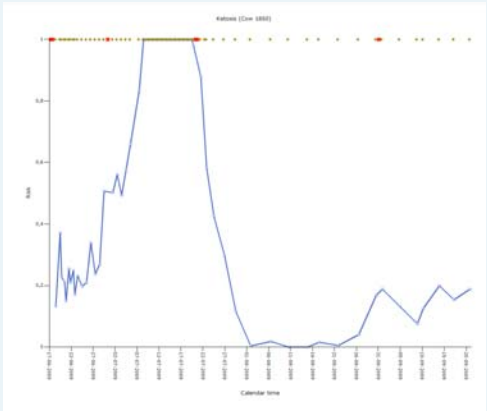
Clinical ketosis alert





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Share of cows with ketosis, Farm 1, over time

- The problem was detected
- A solution was implemented
- 15% went down to 5%

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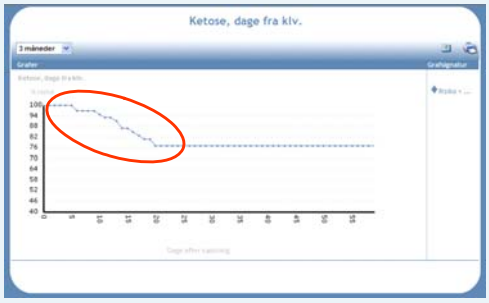
Ketosis frequency indicates how well cows get started!

Farm 1



Farm 2

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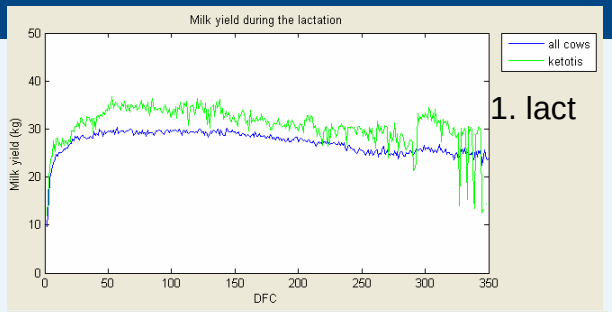
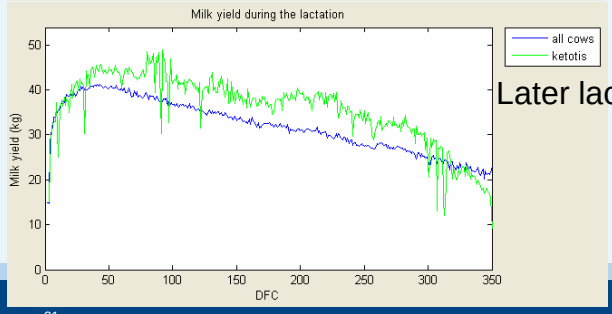
Farm 1, Milk yield for cows with high BHB levels



1. lact



Later lact.

- Cows getting increased BHB concentration in milk has a steeper lactation curve after calving
- If treated/fed correctly then milk yield for these cows will be above average!

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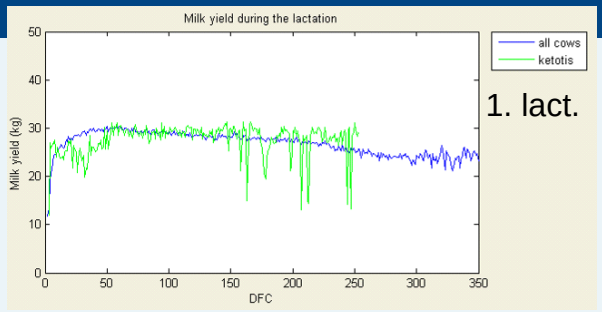
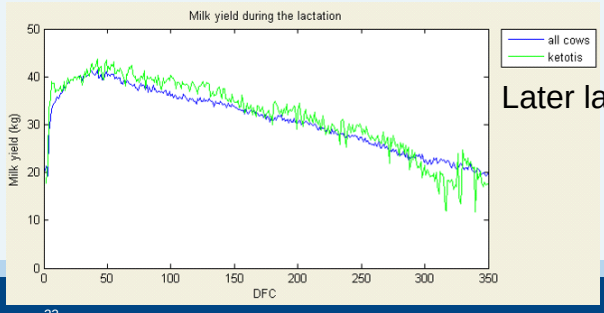
Farm 2, Milk yield for cows with high BHB levels



1. lact.



Later lact.

- Cows with high BHB levels produces on average level!
- TMR is fed which here means that all milking cows are fed the same ratio

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Conclusions Ketosis

- Cows with subclinical and clinical ketosis is readily detected
- By use of Herd Navigator ketosis cows can be treated immediately and remain high yielders
- Herd statistics on type and timing of alerts is basis for an action plan

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Over all benefits

	Farm 1	Farm 2	Farm 3	Farm 4
Empty days at start	112	108	114	125
Empty days now	85	83	84	118
Reduction	**27**	**25**	**30**	**7**

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Statements from users of Herd Navigator

- ”During spring where my focus have been on the field work I still had a very good overview of my herd”
- ”We were surprised about the level of subclinical ketosis and we changed our feeding”
- ”We are now able to monitor the transistion period and help the cow through this period by proactive decisions”
- ”We use the reproductive diagnoses from HN to support the vet.”
- ”We use use the progesterone curves to confirm pregnancy at day 35 from AI”

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Summary

- Reduction in empty days
- Changes in feeding
- Efficient mastitis prevention program
- Monitoring the herd performance
- Early markers for changes in the herd
- Hidden information are transferred to visible signs
- Improved basis for decisions
- Profit improvement varies from 150 – 200 € per cow per year

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Discussion and conclusion

First part:

More than ever international harmonization and standardization are needed in the analytical field to face the globalization of economy and its increasing demand in assessing food products quality and characteristics fairly and equivalently worldwide. This has been the task devoted to analytical method standardization for decades but it has early appeared that working out method standards was only a part of the issue and the optimal use of the method by all the laboratories was needed in addition. Analytical Quality Assurance and accreditation of labs has been one of the possible responses, nevertheless they are individual and somewhat incomplete where laboratories are more and more confronted to others or requested for harmonization where working for the same purposes. The development of international systems, models and structures for analytical harmonization, the assessment and the optimization of analytical performances through collective laboratory participation has become the next issue so as to implement a common analytical language and reach and prove the analytical equivalence everywhere in the world.

ICAR Reference Laboratory Network is one of those systems, specially dedicated to milk recording, and its extension with expansion to every region of the world is needed with this respect for a fluent genetic trade and trustworthy international genetic evaluation.

The best way to make it expand was discussed. It appears that the widest promotion should be made throuth through appropriate means. Publishing the results here presented is one of them. Nevertheless frequency of such publication covering a large period would not be frequent enough. Restriction was made for anonymousness of individual laboratory results that should be respected by law.
It was proposed that regular statistics updates could be made and tables or figures on analytical precision posted on the ICAR website for instance as a control chart for lab network precision with the methods.

The Reference System on Somatic Cell Counting issue was shown as a project development focussed on one major milk element for which the reference method and its application is critical. This is typically a system which involves every aspect of laboratory coordination in networks, as analytical performance evaluation, training, improving and upgrading to the expert level, besides a regular production of reference materials to which reference values are assigned from interlaboratory scheme results.

Second part :

During the last five years, the measurement of fatty acid composition (profile) in milk by Fourier Transform Mid Infra Red spectrometry (FRMIR) has been one major analytical development in milk analysis besides a number of others thus bringing proof, if need was, of its large use spectrum and high interest for milk recording.

Information was given on alternative developments in qualitative analysis through multivariate techniques applied to FTMIR spectra to

- evaluate the animal health and physiological state of cows through a European project of building up a milk FTMIR spectra data base (Optimir project under preparation),

- assess milk “naturality” which is meant as the quality of native milk in absence of adulteration or contamination. In IDF a first step was made successfully with melamine in milk and further developments are expected from FTMIR fingerprints of typical native milk.

It was emphasized on that nutritional quality and milk safety were worldwide issues and characterizing good milk and detecting and tracking back bad milks should be made with standardized technical tools whereas the situation is that where different manufacturers propose FMIR spectrum with different characteristics.

From the various information mentioned, straightforward possible issues were highlighted in

1- defining and standardising the minimum characteristics of FTMIR spectrum as needed for milk analysis,
2- establishing FTMIR correspondence between the various FTMIR milk analysers so as to enable calibration transfer and spectra use through common data bases and collective software.
3- build up an international FTMIR database of standardized FTMIR spectrum that could be used for every purpose after dedicated applications would have been developed.

The last subject on animal health monitoring through on-farm analysis gave information on an innovative joint project now achieved and followed by the implementation of systems at milk producers' in Denmark. Analyses dealt with do not require large precision since they are periodical indicators and can be repeated depending on results obtained. The principle of the method, as based on enzymatic/colorimetric, does not allow implementation in-line for systematic testing and analysis is made after milk sampling. This is an integrated system with help on decision making to the farmer and connectable to larger systems and data bases for herd monitoring.

Conclusion

The meeting was concluded by telling that the presentations showed the two extreme facts of milk recording analytical concern, and the focus of the next years, in the very local analysis for a direct application to herd and milk production on-farm and in the widest concern intending to provide harmonisation of analytical results to make comparable analyses whatever the location and the farm where very dedicated devices are implemented. In between new developments with FTMIR analysis are on to provide the tools to milk producers to drive milk production with taking care of nutritional and technological values of milk, optimizing feeding with benefit on animal health and releasing less nitrogen in environment through animal faeces.

The meeting was shown a success for the quality and richness of subjects, the number of participants and quality of questions and "brainstorming".

The chairman proposed to organize a workshop in Bourg-en-Bresse (France) in 2011 to explore more in depth the today's proposals and to collect new proposals with regard to new parameters worthwhile to be measured in milk for the benefit of milk recording and milk producers.

Christian Baumgartner and Olivier Leray, acknowledged the speakers and invited them for a photograph for souvenir after the session closure, before thanking all the participants and giving them "Rendez-vous!" at the next ICAR session on behalf of ICAR MA SC members.