Second ICAR
Reference Laboratory Network Meeting

Sousse - Tunisia
31 May 2004

MTL WG

ICAR Working Group on
Milk Testing Laboratories

FOREWORD

ICAR Reference Laboratory Network is now in existence for eight years. It was established in order to constitute the basis for an international analytical quality assurance (AQA) system for milk recording. Many country members of ICAR took benefit of the network and the proficiency study schemes implemented for it to develop or improve their national AQA system, whereas others, which had none, may have the opportunity to implement one.
The first meeting of ICAR Reference Laboratory Network held in Interlaken in 2002 was the first opportunity for the members of the network to meet one another and have the possibility to establish links that could enable collaboration and co-operation. 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.

Since 2002, the network has evolved together with new ICAR needs. Indeed it was broadened to laboratories analysing ewe's and goat's milk while new issues about the standardisation of analytical methods for sheep and goat milk occurred and actions were undertaken at the international level of IDF and ISO. Globalisation of economy world wide has urged to take into account technical issues of other species than the cow in the dairy sector with the upgrading of dairy products with regard to quality and hygiene requirements and the economical potential of those types of milk for developing and emerging countries. This fully justifies a focus on analytical concerns with sheep, goat and buffalo milk.

Meanwhile the interest still remains, as a continuous issue, in the development and/or the improvement of those tools that can increase the guarantee of analytical quality, hence the confidence in analytical data worldwide. Thiswas the focus of the presentations in the second part of the meeting. These consisted of an introduction to the new validation system for analytical methods used for milk recording, as an outcome of the Working Group on Milk testing Laboratories (MTL WG), and a review of the results of the international proficiency studies carried out between 1996 and 2003 in ICAR Reference Laboratory Network. Both subjects were chosen to open doors to further thinking and developments.

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, 26 August 2004

Olivier Leray

Chairman of ICAR Working Group
on Milk Testing Laboratories

CONTENTS

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Role and objectives of the network and evolution since 1996

Olivier Leray
Cecalait, Poligny (FR)

The ICAR Reference Laboratory Network was created in 1996 to become the basis of an international analytical quality assurance system for milk recording. It was aimed to serve as a communication tool that can help in harmonising practices in ICAR member countries through standards and recommendations worldwide accepted and a technical medium for providing analytical traceability to routine laboratories and to strive a tightening of the performance of laboratories within and between countries. It is based on a structure with pilot or master laboratories - so-called reference laboratories – that have technical/scientifical experience and knowledge and can supply various technical services or tools for analytical quality assurance (QA) or quality control (QC). One of the prerequisites is to enter the network. Exception is made for those countries which have no more than two routine laboratories and therefore can be connected directly to the international level defined by the network by comparison of national levels where national QC systems are operating for more numerous laboratories. Through the networking principle the intent is also to enable collaboration and co-operation for the benefit of AQA system development in respective countries.

The numbers of laboratories qualified for various scientific/technical mission have increased gradually with the number of members with, during the three last years, a spectacular increase for the evaluation of analytical methods. This is probably related to the development of the international protocol for the evaluation of milk analysers.

At the end of 2003, there are 38 members of 32 countries all involved in cow milk analysis, among which 14 laboratories work also for sheep milk and 14 also for goat milk.

MTL WG

ICAR Working Group on Milk Testing Laboratories

ICAR Reference Laboratory Network

- Second Meeting, Sousse, 31 May 2004

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- Tentative agenda -

- **8.00 :** **Opening - Welcome and introduction of the meeting**
  Presentation of network members
- **8.10 :** **Composition and evolution of the network from 1996**
- **8.20 :** **World wide situation of milk recording in sheep, goat and buffalo**
- **9.40 :** **Analysis of sheep, goat and buffalo milk - Methods, analytical quality and harmonization**
- **10.20 :** **Break (coffee, tea, drinks)**
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- **12.00 :** **Discussion and conclusion of the meeting**
- **12.30 :** **Closure**

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- INTRODUCTION - GENERAL OBJECTIVES -

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

Meeting of ICAR Reference Laboratory Network, 31 May 2004 - ICAR Session Sousse 2004 3

ROLES OF THE NETWORK

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

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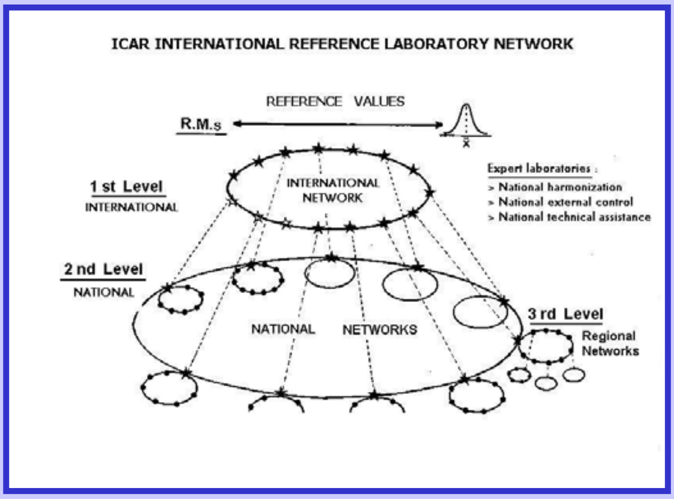
THEORETICAL STRUCTURE



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Missions / activities expected
- Eligibility criteria -

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

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ICAR Reference Laboratory Network
Membership

38 laboratory members from **32** countries

Argentina	(1)	Austria	(1)	Belgium	(2)	Bulgaria	(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	(1)	Lithuania	(1)	The Netherlands	(1)	New Zealand	(1)
Norway	(1)	Poland	(1)	Slovak Repub.	(1)	Slovenia	(1)
South Africa	(3)	Spain	(1)	Sweden	(1)	Switzerland	(1)
Tunisia	(2)	United Kingdom	(2)	U.S.A.	(2)	Zimbabwe	(1)

(n) : number of member(s)

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

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ICAR Reference Laboratory Network
- Evolution since 1998 -

Evolution of the composition and national roles from 1998 to 2003

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

NRTO = National Ring Test Organiser
RMS = Reference Material Supplier
MLCC = Master Laboratory for Centralised Calibrat
TLT = Training in Laboratory Techniques
IAM = Information on Analytical Methods
EAMI = Evaluation of Analytical Methods/Instrumen
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 2001

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

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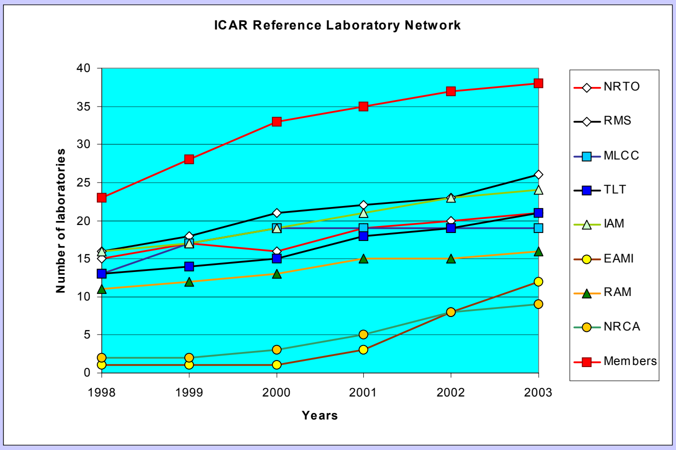
Evolution of membership and missions/activities from 1998 to 2003



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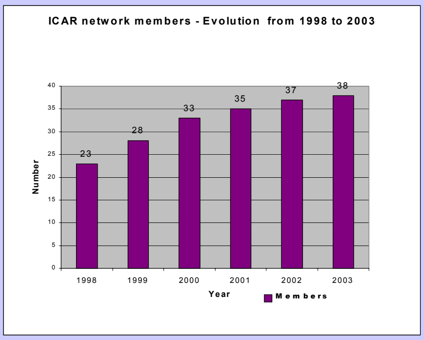

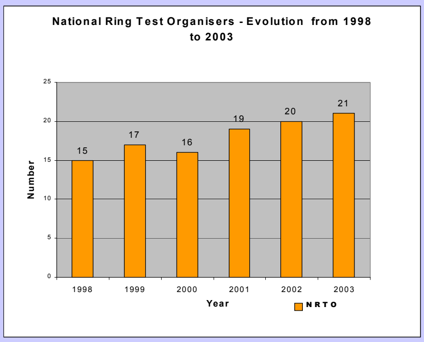

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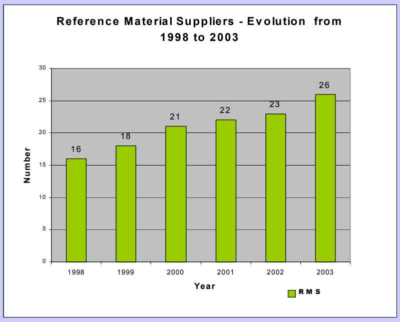

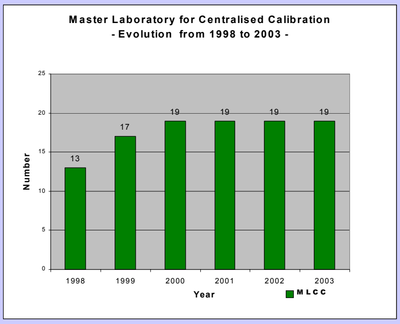

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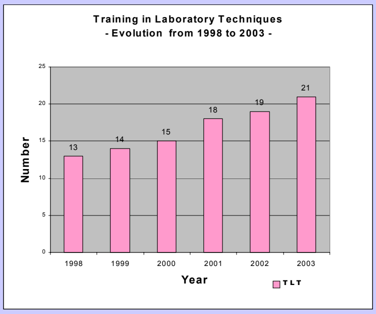

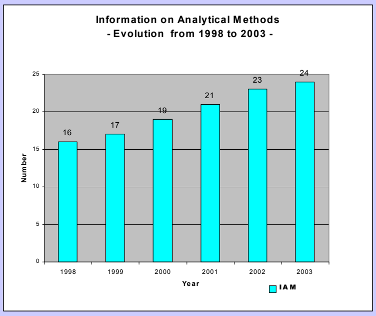

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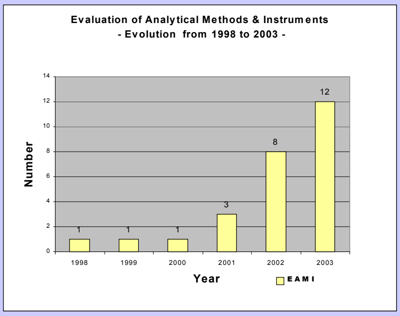

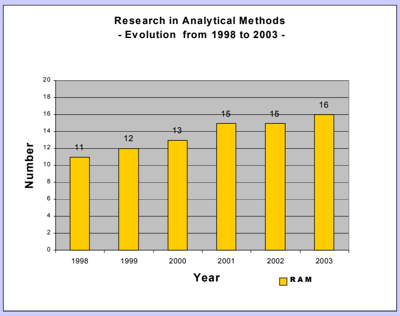

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Worldwide situation of milk recording in sheep

Astruc J.M. [1], Barillet F. , Fioretti M., Gabina D., Gootwine E., Mavrogenis A.P., Romberg F.J., Sanna S.R., Stefanake E.

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Introduction

Specialized milk sheep production is basically located in Europe and in the Middle East, and particularly around the Mediterranean, in harsh local conditions of production. In Europe, Italy, Greece, Spain, Romania, France, Portugal, Bulgaria, Albania (by decreasing order of importance) represent 94 % of the European production (FAO, 2004).
Costs of milk recording in sheep, expressed in production margin per animal, are high compared to that of dairy cattle. Consequently, in most situations, milk recording is not as developed as in cattle. Although original simplified designs, agreed by ICAR, are implemented, the impact of milk recording remains low, especially qualitative recording.
The survey, carried out in the beginning of 2004 by the ICAR working group on milk recording of sheep, will allow us to highlight successively the main features of the development of quantitative milk recording in ICAR countries, the development of qualitative recording, the utilization of qualitative milk recording in breeding schemes. As a conclusion, we will try to draw the prospects in terms of development of milk qualitative recording, in order to open the discussion on an international organization for analytical quality.

Development of quantitative milk recording

The table 1 shows the size of the dairy sheep population and the importance of milk recording in ICAR countries having answered the questionnaire. Two groups of countries may be described: the countries which have a large population (Mediterranean countries) and those with a smaller one, less than 40,000 ewes (Central and Northern Europe). Slovak Republic represents an intermediate situation with a large population (211,000 ewes on the whole).
In the Mediterranean countries, except in France where the impact of milk recording is high (up to 66 % ewes recorded, including D non-official method), the percentage of recorded ewes is usually not higher than 8 % of the whole population. This situation emphasizes the difficulty to implement milk recording in sheep. In Central and Northern Europe (Belgium, Croatia, Czech Republic, Germany, Slovak Republic, Slovenia, Switzerland), milk recording is carried out in small flocks and represents few recorded ewes, even though the percentage of recorded ewes is quite high.

Development of qualitative milk recording

Conversely to dairy cattle, qualitative recording (which entails taking representative samples in order to analyse fat and protein content, SCC …) is optional in official milk recording of sheep, as established in the ICAR guidelines (ICAR, 2003), because it becomes cost-effective to implement only when selection on milk yield is efficient. Moreover qualitative recording is often technically difficult to set up in large flocks.
Consequently, as shown in the table 1, the number of ewes in qualitative recording is small and represents a fraction of the ewes in quantitative milk recording :

- Some countries don't implement qualitative recording. In 2003, among 14 countries where milk recording is carried out, only 10 countries practice a qualitative recording.
- Among these countries, qualitative recording is not implemented in all breeds and all flocks. For example, in Italy, qualitative recording only concerns Sarda breed.
- As a way of simplification (Astruc and Barillet, 2004), in several countries, only some parities are tested (parity 1 or parities 1 and 2). Furthermore, in France, a part-lactation sampling method (efficient enough for genetic purposes) has been conceived (it is also used in Italy in Sarda breed) consisting in recording only the most representative part (from a genetic point of view) of the lactation.

On the whole, the test-days where samples are picked up may be very limited. As an illustration, in France, 1,688,900 individual test-days were recorded in 2003 (quantitative recording). 278,800 had a sample analysed, representing only 16.5 %.

The type of analysis carried out in the laboratories varies among the countries, and sometimes within a country from a breed to another one. The table 2 summarizes the current situation.

- Fat and protein content are analysed in all the cases, somatic cell count in 12 countries out of 13. Hygienic quality of the milk is a more and more important issue (price of the milk, perspective of EU regulation on somatic cell count in sheep and goat milk).
- Lactose is analysed in countries of Central Europe (Croatia, Czech Republic, Slovak Republic, Slovenia) and Spain.
- Manchega and Latxa breeds in Spain analyse dry matter.
- Finally, urea is analysed only in Switzerland and Germany.

Utilization of qualitative milk recording in breeding schemes

First of all, it must be kept in mind that on-farm collective breeding schemes implemented at the population level are not widely developed in dairy sheep, whatever the objective and criteria of selection, except in France, Italy and Spain. About analytical aspects in the selection criteria in dairy sheep, we can point out that milk composition, when recorded, is more and more often included in the selection criteria. Inversely, somatic cell count, although recorded, is not included in the selection criteria, excepted in France. Lactose, dry matter and urea are never included in selection criteria.

Conclusion

Although the impact of milk recording in dairy sheep remains globally limited in the countries with a large population of dairy sheep, its development is slowly increasing. Qualitative milk recording, though implemented in more and more countries, often concerns a fraction of the flocks and among them a fraction of the ewes. The cost of qualitative recording currently prevents from developing it on a large scale. Although it is difficult to forecast the development of sheep milk analysis in the next future, it is likely that the analysis of somatic cell count will increase with the growing concern of sanitary and hygienic problems.
The question of the quality of the analysis (fat and protein as well as somatic cell count) must be arisen. Analysis of sheep milk is often done in laboratories where a large number of analyses of cow milk are carried out. If the specificities of sheep milk are not taken into account (the fat and protein contents of sheep milk are about twice as high as in cow milk), the results might be biased and/or not accurate. Are the methods used for cattle compatible with sheep milk ? Do the laboratories implement the right standardization and calibration ? These questions might justify an international standardization of method of analysis and an organisation for the analytical quality.

Table 1. Size of population of dairy sheep, impact of quantitative and qualitative recording in ICAR member countries .

Countries	Size of population	Quantitative recording	Qualitative recording	
		Number of recorded ewes (official milk recording)	Yes/No	Number of recorded ewes
Belgium		277	No	-
Croatia	33,000	2,920	Yes	1,733 (59%)
Czech Republic	473	204	Yes	All
England / Wales		692	No	-
France	1,395,000	305,143 (*)	Yes	90,279 (30%)
Germany	20,000	1,193	Yes	All
Greece	8,732,000	7,885	No	-
Israel	46,200	14,975	No	-
Italy	6,150,000	478,992	Yes	30,238 (6%)
The Netherlands	4,000	1,156	Yes	All
Portugal	105,000	21,000	No	-
Slovak Republic	216,000	17,846	Yes	All
Slovenia	6,300	1,704	Yes	All

Spain	2,361,000	188,197	Yes	71,045 (38%)
Switzerland	8,000	2,800	Yes	All
Tunisia	25,000	2,393	Yes	All

(*) in addition, 616,337 ewes are recorded with D method (non-official milk recording) without qualitative recording

Table 2. Type of milk analysis in countries where qualitative recording is implemented .

Milk analysis	Countries
Fat, protein, somatic cell count, lactose	Croatia, Czech Republic, Slovak Republic, Slovenia
Fat, protein, somatic cell count, lactose, dry matter	Spain (Manchega and Latxa breeds)
Fat, protein, somatic cell count, lactose, urea	Switzerland, Germany
Fat, protein, somatic cell count	France, Spain (Churra and Castellana breeds), the Netherlands
Fat, protein	Italy (Sarda breed)

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Astruc JM., Barillet F., Fioretti M., Gabina D., Gootwine E., Mavrogenis AP., Romberg FJ., Sanna SR., Stefanake E., 2004. Report of the working group on milk recording of sheep. Proc. 34th Biennial Session of ICAR, Sousse, Tunisia, 31 May-3 June 2004.

FAO, 2004. FAOSTAT statistical database. http://www.fao.org/.

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WORLDWIDE SITUATION OF MILK RECORDING IN SHEEP

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* Institut de l'Elevage - Toulouse, France
** **M**embers of the WG on milk recording of sheep

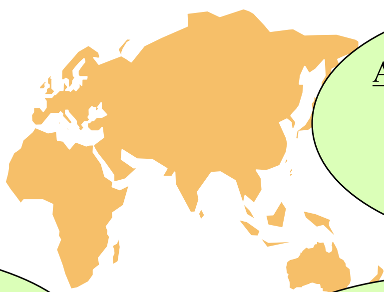
ICAR Reference Laboratory Network
Sousse – Tunisia, 31 May 2004

Worldwide milk sheep production

FAO estimations 2002 : 7,764,000 t

EUROPE : 2,742,000 t

Italy, **Greece**, Spain, Romania, France : 85 %



ASIA : 3,382,000 t

China, Turkey, Syria, Iran, Afghanistan, Iraq : 85 %

AFRICA : 1,605,000 t

Sudan, Somalia, Algeria : 69 %

OCEANIA, AMERICA : 35,000 t

small production
emerging countries ?

Survey on milk recording of sheep

- Several surveys carried out in the framework of ICAR (organized by the WG on milk recording of sheep)
  1988 - 1996 – 1998 – 2000 – 2002 - 2004

- PURPOSES :
  - Situation of milk recording in dairy sheep
  - Follow application of ICAR guidelines
  - Stimulate new developments (among them MILK ANALYSIS)

ICAR & non ICAR members : 24 answers / 45 questionnaires sent

Sheep milk recording (2003)

⇒ 1,050,000 females reported to be submitted to official milk recording in 16 countries (Europe + Israel + Tunisia).

⇒ France, Italy, Spain = 93 %

Mediterranean countries

- Large population of dairy sheep.
- Small impact of milk recording (4% on average)

France = exception

- 66% ewes milk recorded (22% method AC, 44% method D)

Sheep milk recording (2003)

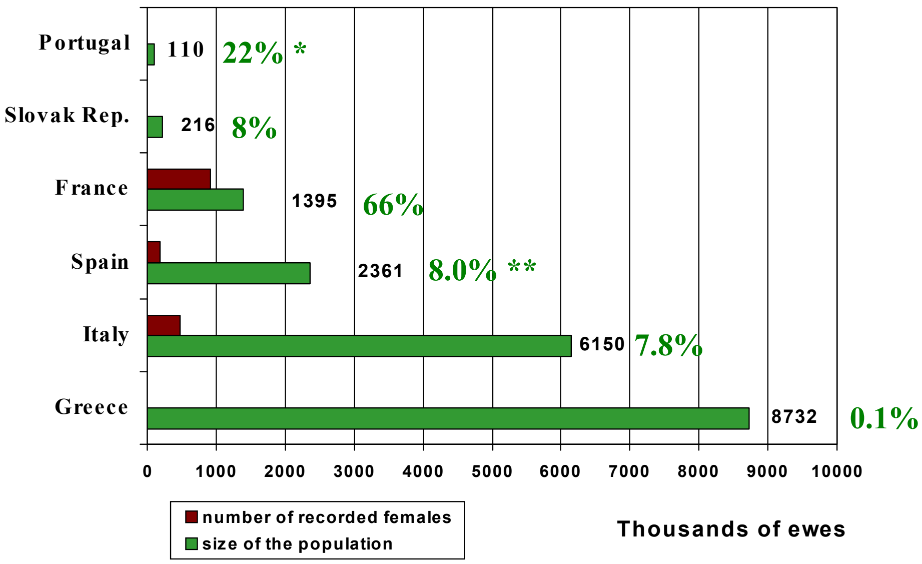
Central and Northern Europe

- Small populations of dairy sheep
- High impact of milk recording (18%)
- Large population in Slovak Republic

Balkan

- Dairy sheep farming tradition but no answer from the questionnaire

Sheep milk recording in countries with more than 100,000 ewes (2003)

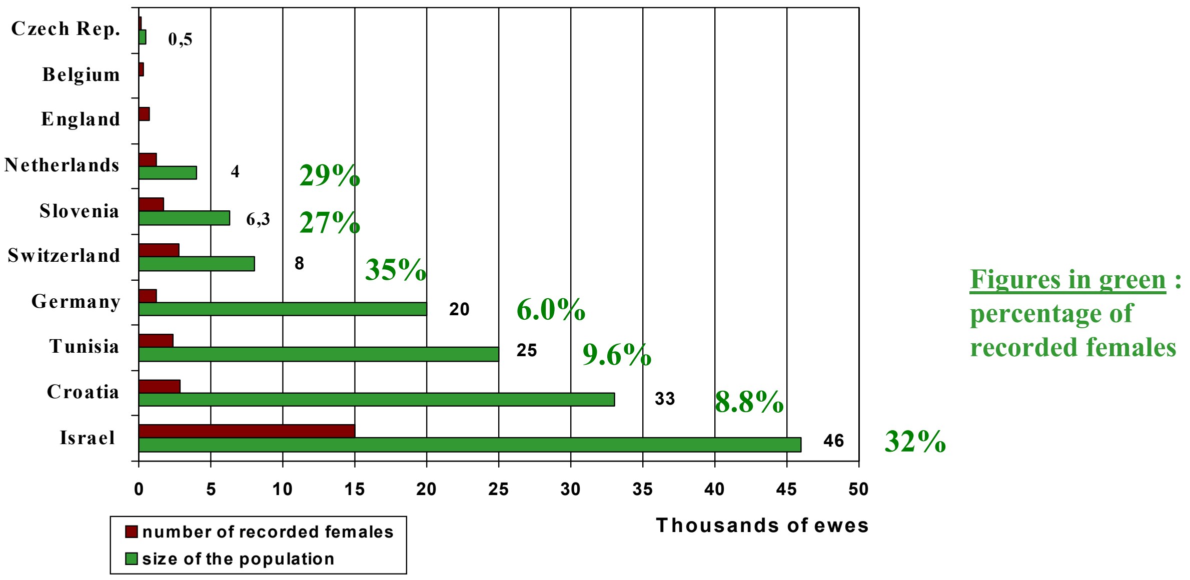


* Serra da Estrella & Mondegueira breeds only

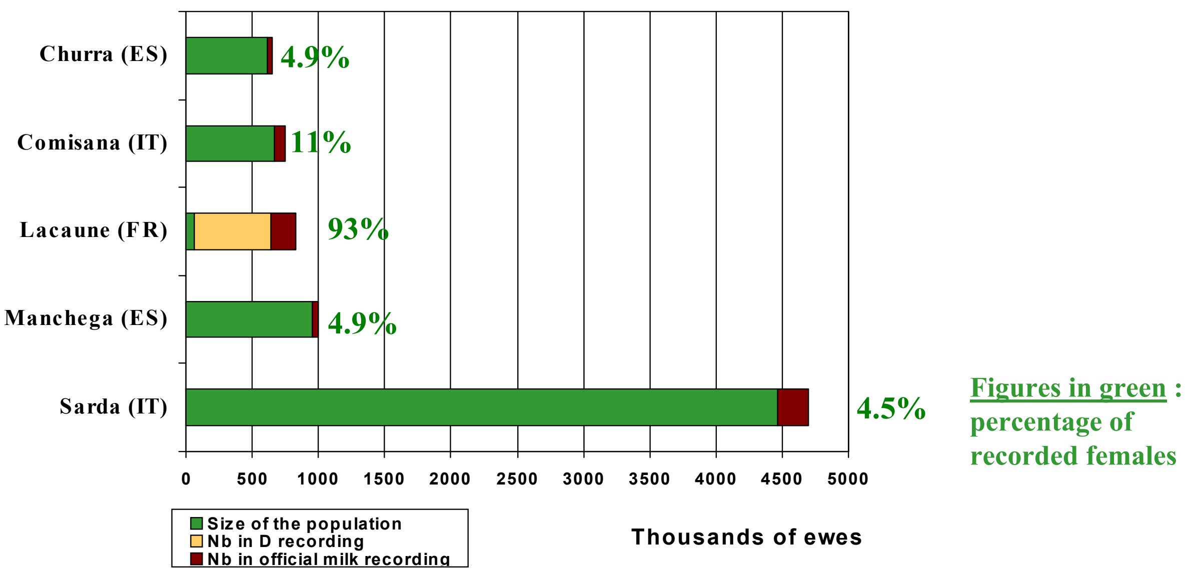
** Local breeds only

Figures in green : percentage of recorded females

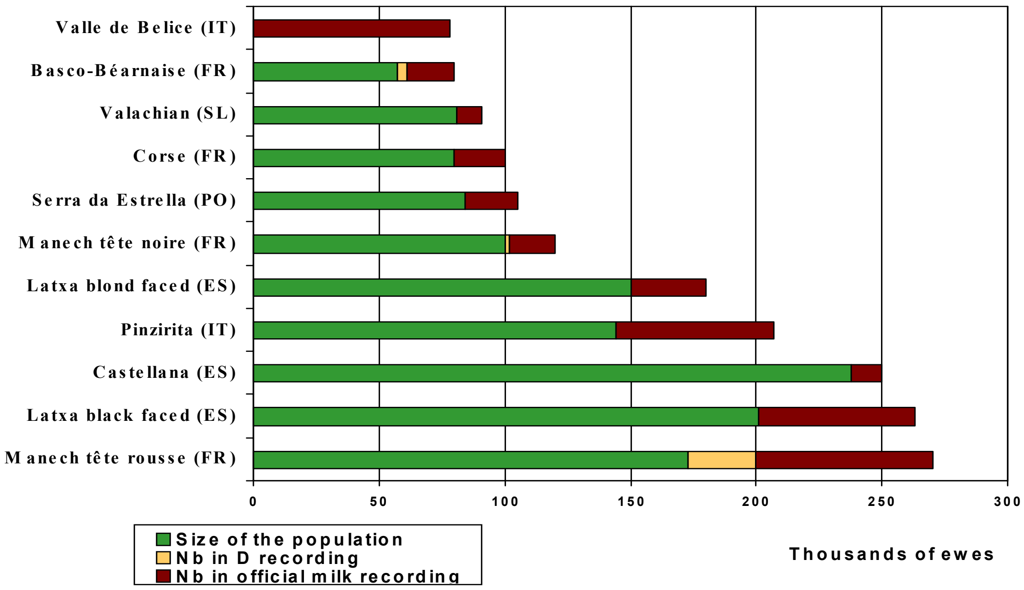
Sheep milk recording in countries with less than 50,000 ewes (2003)



Sheep milk recording in breeds with more than 500,000 ewes (2003)



Sheep milk recording in breeds with less than 300,000 ewes (2003)



General context of milk recording in sheep

Costs of milk recording in sheep, expressed in production margin per animal, are high compared to that of dairy cattle (especially for qualitative recording)

Large flocks (200-500 ewes)

High milking speed (3 min per ewe)

↗ Number of technicians

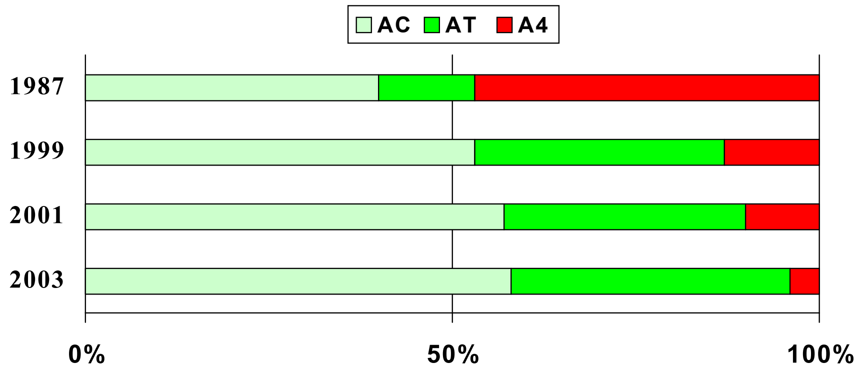
↗↗ Number of analysis

Qualitative test = facultative recording

Necessity of simplification

Simplification of Milk recording

Milk yield : increasing use of simplified (AT or AC) methods



% simplified methods

53%

87%

90%

96%

Simplification of Milk recording

Milk quality (optional disposition) : part-lactation sampling within AC method implemented only in France and Italy (Sarda breed)

AC method : record of 1 of the 2 milkings

Parities 1 (& 2) ⓧ ⓧ ⓧ X X X

A4 method, all ewes → For 100 ewes : 100 x 12 = **1200 samplings**

Part-lactation sampling within AC method, parities 1 & 2 → For 100 ewes : 60 x 3 = **180 samplings (15 %)**

Countries with samplings / analysis

11 countries / 16

England

The Netherlands

Germany

Czech Rep.

Belgium

Switzerland

Slovak Rep.

France

Slovenia

Spain

Italy

Croatia

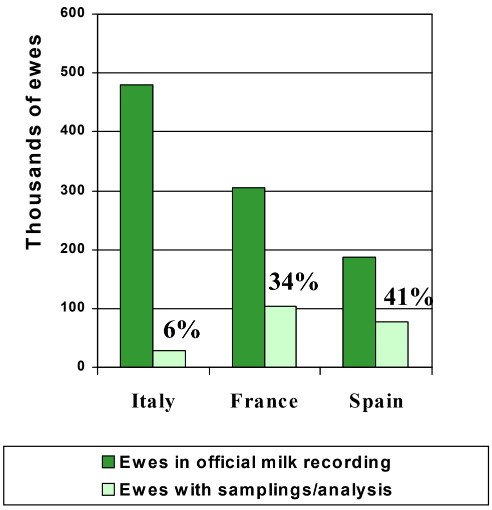
Greece

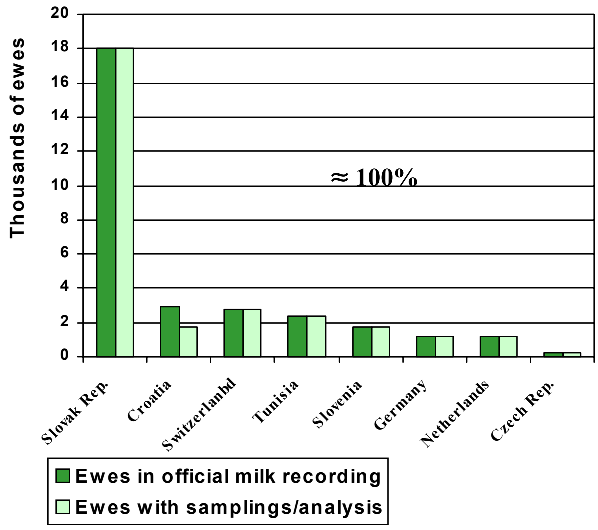
Portugal

Tunisia

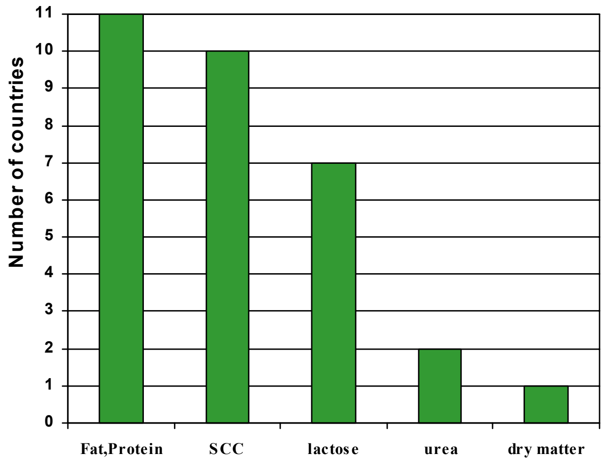
Israel

Part of the ewes in official milk recording submitted to qualitative recording





Type of analysis done by countries



SCC : all except Italy

Lactose : Croatia, Czech, Germany, Slovak, Slovenia, Spain, Switzerland

Urea : Germany, Switzerland

Dry matter : Spain

Breeding schemes and selection criteria

Few countries with progeny-test and AI (France, Italy, Spain)

	Number of AI progeny-tested rams	AI (2003)	Number of breeds	Selection criteria
FRANCE	678	514,000	5	Lacaune : FY+PY+0.2P%(+SCS) Pyrenean : FY+PY Corse : MY
ITALY	50	19,000	1	Sarda : MY
SPAIN	270	73,000	4	All breeds : MY except Churra : MY + P%

Meters and Jars

- **Important variety of meters and jars**
  - **- Jars or meters for dairy cattle used for sheep**
  - **- Specific jars or meters**
- **None of them agreed by ICAR**
- **Some of them without sampler**

Concerns and Perspectives

⇨ **Breeders' concerns**

High costs of qualitative recording (recording + analysis)

↳ ▪ Necessity to implement simplified methods

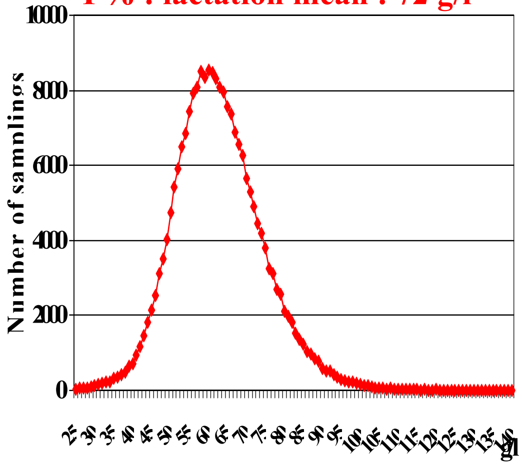
Increasing interest for somatic cell counts (sanitary concern, price of the milk, EU regulations)

We know today's activity. What about to-morrow's demand ?

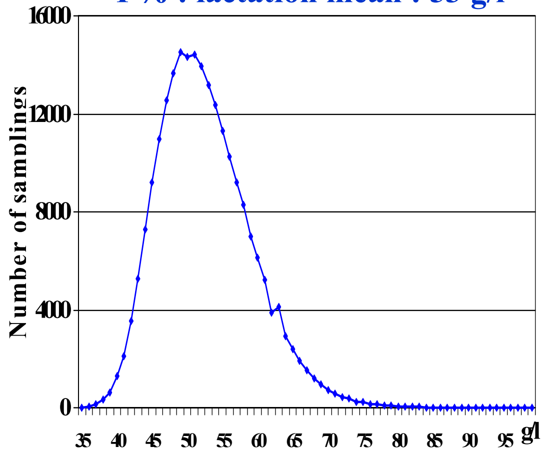
Milk sheep's contents are high

Distribution of fat (red) and protein (blue) content from morning milkings in Lacaune breeds (ewes of parities 1 and 2, data from 2003)

F% : lactation mean : 72 g/l



P% : lactation mean : 53 g/l



Concerns and Perspectives

⇨ **Laboratories' concerns**

❖ Fat & protein contents of sheep milk are almost twice as high as the one of cow milk

❖ Sheep milk analysis often represents a small part of the activities of a laboratory compared to dairy cattle's activity.

↳
- Are the devices adapted for milk sheep ?
- Standardization of reference methods
- Do the laboratories always implement specific calibration of the devices ?

Worldwide situation of milk recording in goat

Drago Kompan

University of Ljubljana, Biotechnical faculty, Zootechnical Department Groblje 3, 1230 Domzale, Slovenia

Goats have played a role in food culture since time immemorial with ancient cave paintings showing the hunting of goats. They are also one of the oldest domesticated animals since the herding of goats is thought to have evolved about 10-11 thousand years ago.
Goat milk and the cheese made from it were revered in ancient Egypt and were also widely consumed by the ancient Greeks and Romans. Goat milk has remained popular throughout history and still is consumed on a more extensive basis worldwide than cow's milk. Perhaps more people drink goat milk than cow milk.
The situation in the goat sector in the last 20 years is increasing with 60% in the size of goat stocks (head) and 50 % in goat milk production. This is one of the greatest increases in livestock sector worldwide.
The situation on milk recording is also very diverse. They are many local breeds, well adapted to local condition and traditional rearing. The majority of goat milk production on ICAR member countries is based in France, Greece, Italy, Switzerland, Norway and some small number of milk goat breeds in UK, Netherlands, Germany, Slovakia, Croatia and Slovenia.
Milk yield and milk composition of different breeds is very diverse. The Toggenburg breed from UK has 1339 kg milk yield per lactation, whereas for Garganica from Italy 134 kg total milk yield is reported. The difference also pertains to milk composition, on lenght of lactation and flock size. Various simplified milk recording methods exist between countries.
The percentage of recorded goats is very diverse between countries (0.14 % – 87 %).
Official milk recording (A4, AT4 methods) is used in a number of countries, B method is used only in Croatia and Germany, method E is used in Germany (1 %) whereas the majority of the countries implement AT recording.
A4 method is being progressively replaced by two simplified designs for milk yield (AT or B4). Our survey confirms this trend and clearly shows that simplification of milk yield recording has widely spread among ICAR countries.

Network MTL

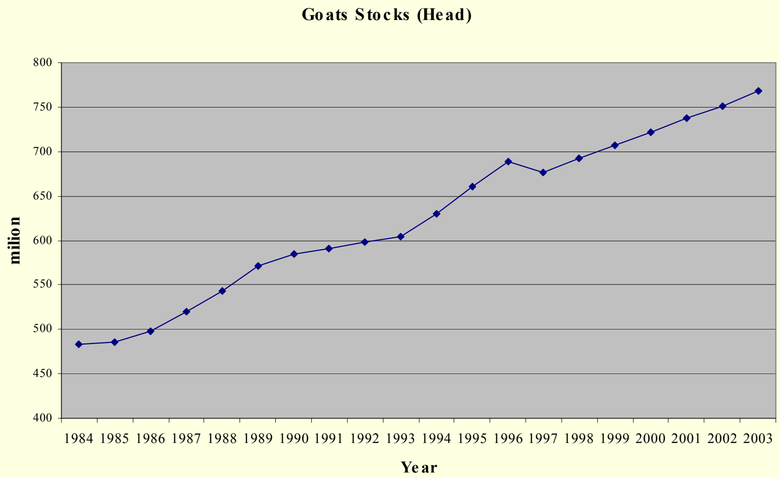
Worldwide situation of milk recording on Goat

Drago KOMPAN
University of Ljubljana

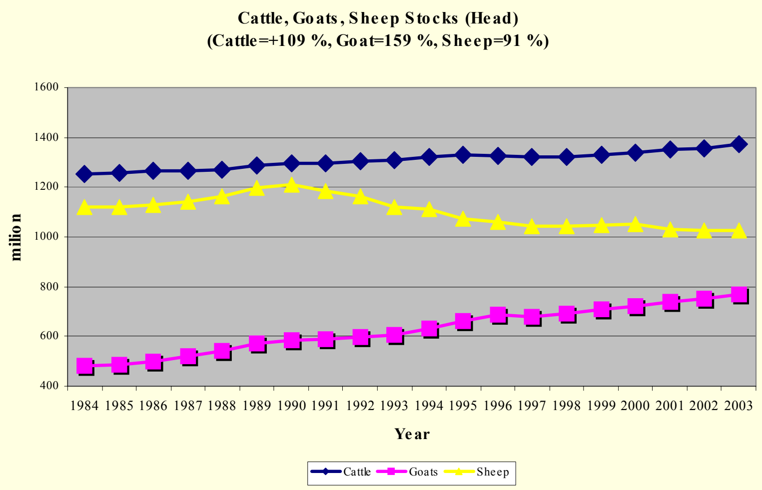
SLOVENIA

Goats *Stocks (Head)*	Year			
	2000	2001	2002	2003
European Union (25)	12,572,172	12,027,770	12,089,252	12,377,904
Reg Off Near East	175,010,822	177,903,587	181,955,072	186,936,147
Reg Off Asia & Pacific	421,271,028	432,404,641	441,236,327	453,415,320
Reg Office Africa	197,104,056	202,388,934	205,057,533	206,572,909
Reg Office Europe	24,287,530	23,051,833	22,787,113	23,321,464
Reg Office Lat.Amer. & Cariben	34,655,490	34,670,480	35,120,249	34,846,491
South America	22,854,297	22,818,519	22,256,737	22,068,000
South Asia	211,365,344	214,008,984	218,890,258	218,970,000
Southern Africa	5,121,369	5,091,060	5,404,602	5,102,000

World situation on goat sector in last 20 years (+60%)



Comparison on last 20 years



Average milk yield per recorded does

COUNTRY BREED	Average milk yield per recorded does in kg (length in days) [a = TMY(milking without suckling) / b = TMM (total milk yield)]			Number of flocks (goat)
	yearlings (12-18 months)	Adults (>18 months)	all does	
UK				
ANGLO NUBIAN	905 [a]	1071 [a]	1038 [a]	4 (594)
ALPINE	748 [a]	1042 [a]	983 [a]	
SAANEN	976 [a]	950 [a]	962 [a]	
TOGGENBURG	-	1339 [a]	1339 [a]	
OTHERS	1129 [a]	1034 [a]	1070 [a]	
TOTAL	978 [a]	994 [a]	987 [a]	

COUNTRY BREED	Average milk yield per recorded does in kg (length in days) [a = TMY (milking without suckling) / b = TMM (total milk yield)]			Number of flocks (goat)
	yearlings (12-18 months)	Adults (>18 months)	all does	
	yearlings (12-18 months)	Adults (>18 months)	all does	
SLOVAK REPUBLIC				
WHITE GOATS	-	509 [b]	508,9 [b]	17 (607)
ITALY				
MALTESE	242........[b]	339........[b]	326........[b]	68 (3150)
GARGANICA	117........[b]	136........[b]	134........[b]	6 (242)
GIRGENTANA	282........[b]	345........[b]	336........[b]	12 (380)
BIONDA DELL'ADAMELLO	-	329........[b]	329........[b]	6 (132)
SAANEN	373........[b]	605........[b]	521........[b]	182 (7876)
CAMOSCIATA DELLE ALPI	343........[b]	525........[b]	478........[b]	186 (6312)
FRISA	271........[b]	356........[b]	343........[b]	5 (276)
SARDA	162........[b]	229........[b]	221........[b]	58 (4592)
DERIVATA DI SIRIA	121........[b]	413........[b]	409........[b]	38 (1287)
TOGGENBURG	382........[b]	270........[b]	326........[b]	2 (2)
ARGENTATA DELL'ETNA	138........[b]	187........[b]	185........[b]	28 (531)
JONICA	158........[b]	253........[b]	248........[b]	18 (980)
VALLESE	106........[b]	195........[b]	173........[b]	1 (12)
VERZASCHESE	185........[b]	331........[b]	321........[b]	7 (191)
OROBICA	184........[b]	282........[b]	277........[b]	60 (851)
ROCCAVERANO	246........[b]	390........[b]	363........[b]	15 (368)
MESSINESE	137........[b]	182........[b]	177........[b]	63 (5696)
LARIANA	-	198........[b]	198........[b]	-

COUNTRY BREED	Average milk yield per recorded does in kg (length in days) [a = TMY (milking without suckling) / b = TMM (total milk yield)]			Number of flocks (goat)
GERMANY	yearlings (12-18 months)	Adults (>18 months)	all does	
WEIßE DEUTSCHE EDELZIEGE			764 (240) [a]	52 (1481)
BUNTE DEUTSCHE EDELZIEGE			626 (240) [a]	125 (3026)
THÜRINGERWALDZIEGE			767 (240) [a]	33 (221)
TOGGENBURGERZIEGE			732 (240) [a]	6 (100)
ANGLONUBIER			621 (240) [a]	6 (48)
TOTAL			681 (240) [a]	
NORWAY NORWEGIAN DAIRY GOAT			576 [a]	450 (38000)
CROATIA	345 (187) [b]	519 (214) [b]	450 (199) [b]	(2549)
FRENCH ALPINE (AT)	291 (175) [b]	424 (188) [b]	379 (183) [b]	(654)
FRENCH ALPINE (B4)	247 (159) [b]	401 (178) [b]	378 (174) [b]	4 (71)
GERMAN IMPROVED FAWN	561 (208) [b]	704 (224) [b]	666 (220) [b]	9 (224)
GOATS				
SANNAN				

COUNTRY BREED	Average milk yield per recorded does in kg (length in days) [a = TMY (milking without suckling) / b = TMM (total milk yield)]			Number of flocks (goat)
FRANCE				
ALPINE			731	2139
SAANEN			745	
GREECE				
SKOPELOS			359	
KARYSTOU			82	
LOCAL			140	
SLOVENIA				
SAANEN			523(246) [b]	10 (368)
ALPINE			419(244) [b]	34 (1396)
LOCAL			226(203) [b]	7 (101)

Milk yield and reference length

COUNTRY BREED	Average milk yield per recorded does in kg (length in days) [a = TMY / b = TMM / c=TSMM] ref = reference length in days			Number of flocks (goat)
SWITZERLAND	yearlings (12-18 months)	Adults (>18 months)	all does	
SAANEN	600 (263) [a]	828 (272) [a]	778 (270) [a]	(3297)
APPENZELL	522 (255) [a]	754 (264) [a]	694 (262) [a]	(434)
TOGGENBURG	555 (259) [a]	793 (271) [a]	738 (268) [a]	(1183)
ALPINE (CHAMOISEE)	522 (252) [a]	735 (265) [a]	687 (262) [a]	(3245)
BÜNDER STRAHLEN	355 (219) [a]	576 (239) [a]	539 (235) [a]	(456)
NERA VERZASCA	-	439 (207) [a]	439 (207) [a]	(334)
PFAUENZIEGE	322 (248) [a]	511 (238) [a]	475 (236) [a]	(202)
NUBIAN	780 (300) [a]	**891 (300) [a]**	828 (300) [a]	(14)
TOTAL	546 (255)	749 (263) [a]	705 (262) [a]	(9176)

Milk yield and reference length

COUNTRY BREED	Average milk yield per recorded does in kg (length in days) [a = TMY / b = TMM / c=TSMM] ref = reference length in days			Number of flocks (goat)
ITALY	yearlings (12-18 months)	Adults (>18 months)	all does	
MALTESE	242........[b]	339........[b]	326........[b]	68 (3150)
GARGANICA	117........[b]	136........[b]	134........[b]	6 (242)
GIRGENTANA	282........[b]	345........[b]	336........[b]	12 (380)
BIONDA DELL'ADAMELLO	-	329........[b]	329........[b]	6 (132)
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LARIANA	-	198........[b]	198........[b]	-

Recorded goat number

UK	594
SLOVAKIA	607
SLOVENIA	1.865
CROATIA	3.498
GREECE	6.189
GERMANY	6.714
SWITZERLAND	13.848
ITALY	26.066
NORWAY	38.000
FRANCE	320.000

Milk recording equipment used

USE OF YARS (volume)

MANUFACTURE:

- ALFA LAVAL
- ROYAL
- WESTFALIA
- MISURATORE ITALIANA
- MIBO
- "CARTEL" GERMANY

Milk recording equipment used

USE OF MILK METERS

MANUFACTURE:

TRU TEST (type: HI, FV, FD-5G)

Measurement

Volume

Weight

Worldwide situation of milk recording in buffalo

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Introduction

Purpose of this paper is to examine the possibility of expansion of the ICAR reference lab WG in the area of buffaloes. A preliminary outlook on the distribution of buffalo and the assessment of the already established recording systems is therefore a preliminary step.
The idea to include buffaloes in the activities of ICAR was born 10 years ago. During those years, ICAR activity has focused on the following items:

1. knowledge of the geographical location of buffaloes, i.e. where they are mainly concentrated and where they have an economic importance at least equal, or higher, than dairy cows;
2. verify the possibility to promote milk performance recording in buffalo;
3. propose simplified recording procedures for low-input production systems, being aware that buffaloes are reared in the developing countries.

At each ICAR meeting since 1996, statistics were presented on population size and numbers of milk recorded buffaloes in each country. I will here present an updated overview with statistics referring to the years 2000-2003, where population numbers were taken from FAO Yearbooks and recording data from the ICAR surveys.

Geographical distribution

Country	Dairy buffaloes (adult females)
India	46,000,000
Pakistan	10,650,000
Egypt	2,400,000
Nepal	836,500
Iran	208,200
Azerbaijan	150,000
Italy	125,000
Turkey	58,806
Brazil	52,000
Romania	42,300
Bulgaria	4,980
China (only dairy)	2,900
Greece, Macedonia, Albania, Switzerland, Germany, UK	Few hundreds

In the above table, countries are listed based on the population size. India and Pakistan clearly stand out at world level. But also Egypt and Nepal leave the remaining countries a good deal behind. I know that this rank has not much meaning because we cannot compare huge countries like India with small countries like Nepal or Bulgaria, but we must speak in terms of countries if we want to plan any future activity or cooperation project.
More useful and mathematically more correct is to examine the ratio dairy buffaloes out to total dairy females (cattle+buffaloes); this parameter gives in fact the idea of the economic importance of buffalo milk locally.

Country	Dairy cows	Dairy buffaloes (females)	Percentage of dairy buffaloes out of total dairy females (cattle+buffaloes)
Pakistan	4,366,000	10,650,000	71.0
Egypt	1,253,000	2,400,000	66.0
India	35,500,000	46,000,000	56.4
Nepal	828,000	836,500	50.2
Azerbaijan	820,000	150,000	15.0
Italy	2,169,000	125,000	5.4
Iran	3,543,000	208,200	5.0
Romania	1,600,000	42,300	2.6
Bulgaria	430,000	4,980	1.1
Turkey	5,700,000	58,806	1.0
Brazil	27,800,000	52,000	0.19
China (only dairy)	4,633,000	2,900	0.06

In the above table, in which this parameter was considered, Pakistan and Egypt get the best position (more buffaloes than cows); India and Nepal have around the same number of buffaloes and cows; but the remaining countries have less than 6 percent, except Azerbaijan (15%).

Milk performance recording

The third important parameter is the extension of milk recording, shown in the following table as percentage of recorded buffaloes out of total adult female population.

Country	Total milk recorded buffaloes	% recorded out of total buffalo population
Italy	35,755	28.6
Bulgaria	425	8.5
Iran	13,236	6.3
India - Gujarat	57,500	2.3
Pakistan		
- Punjab	6,000	0.08
- North West Frontier Provinces	26,000	2.5
Egypt	24,000	1.0
Brazil	500	1.0
Turkey	200	0.34
Azerbaijan	100	0.06
Nepal	400	0.05
Romania	-	-

Except for Italy (28.6 % of milk recorded buffaloes) but also for Bulgaria and Iran (8.5 and 6.3%), the remaining countries fall below 1%. However, Gujarat must be mentioned and kept separate from the whole of India because it registers 2.3 % of recorded buffaloes, which means a good number of animals and work. The Gujarat case is also very interesting from a management point of view. It is the only case of buffalo milk recording which is managed by cooperatives of private farmers. In all other cases, in fact, the governments, through the Ministries of Livestock Affairs, Agriculture or other, are directly responsible of the implementation and management of the milk recording activity and give at least part of the funds for the regular running. A few words are therefore necessary to depict the Indian system, as a well organized

running programme for animal recording and selection. Dairy cooperatives account for the major share of processed liquid milk marketed by 170 Milk Producers' Cooperative Unions, which federate into 15 State Cooperative Milk Marketing Federations. The National Dairy Development Board is a body corporate that was created in 1987 by merging two older societies. It covers the whole of India and was created to promote, finance and support producer-owned and controlled organizations. NDDB's programmes and activities seek to strengthen farmer cooperatives and support national policies that are favourable to the growth of such institutions. Fundamental to NDDB's efforts are cooperative principles; NDDB 's programmes and activities seek to strengthen the functioning of Dairy Cooperatives, as producer-owned and controlled organizations. NDDB supports the development of dairy cooperatives by providing them financial assistance and technical expertise. The Dairy Cooperative Network operates in over 285 districts, covers nearly 1,031,281 village level societies and it is owned by nearly 11 million farmer members. The National Dairy Development Board (NDDB) was the promoter and is the executer of the whole recording and selection activity in India and the most concrete and successful results of buffalo improvement have been obtained in Gujarat.
In order to identify what buffalo realities might be targeted by the lab WG, we are compelled to examine the above presented figures from different positions, and to consider each of them separately.

ICAR membership

First, what countries are already ICAR members and how do they behave with regard to buffalo recording systems?
I remind you that not in all cases, although in most, the government of the country becomes a member of ICAR. In this case it is assumed, but can be untrue in practice, that ICAR membership include all milked animals, therefore ICAR regulations for milk recording are accepted by the country for all milked animals.
Italy, Bulgaria and Gujarat (India) are ICAR members and have a long tradition of buffalo recording as well. In these countries buffalo milk recording is performed following the same rules as for the dairy cows (A4 method). This is to refute and discredit the common opinion that buffalo is a difficult and wild livestock to be handled as regards to milk recording. On the contrary, it is exactly like a dairy cow, and this is proven by the fact that in many Italian farms, like the station where I work, Friesian cows and buffaloes are kept in two equal paddocks and are milked in the same milking parlour. Differences in lactation physiology (in fact buffalo has generally a shorter lactation duration compared to cows) and in milk composition (but also the Jersey cows produce high butterfat mik) do not deserve to treat buffaloes as a different livestock. Germany, UK and Switzerland, where several buffalo herds are now established, will likely join the ICAR milk recording system also for buffaloes as soon as the herds will get interested in the selection activity. Turkey also is an ICAR member although buffaloes are milk recorded only at research level, but buffalo in Turkey do not have an economically important status. Egypt has a recently established official recording system and has joined ICAR a few years ago. In Egypt buffaloes are as important as cows, therefore both a deeper involvement of this country in ICAR activities and an increasing number of recorded animals is expected.
Conclusionn 1 is that all ICAR member countries will appreciate and take advantage of any future proposal in the matter of buffalo milk analyses.

Long tradition of animal recording outside ICAR

The second aspect to be examined is the one related to the countries that believe in the usefulness of milk recording and have a long history of milk performance recording and selection for buffaloes. However, they have not shown any interest in becoming ICAR members. This is the case with Pakistan and Iran, where, on one side, buffaloes are considered as economically important of even more important than cows, and where milk recording activity and selection have been performed for many years, also involving small-holders and private farmers, and registering all information at central level, and make use of them for the selection activity. Representatives of those countries have participated in various ICAR meetings, but although their recording systems are already fully harmonized with the ICAR systems, no issue for involvement in ICAR has yet been found. The laboratory network might find in those countries many opportunities for cooperative projects.

Developing countries

The third aspect to be examined is the one of the countries where buffalo is very important economically, but the unawareness of the importance of recording as well as the lack of financing forbid either any activity of cooperation with ICAR or the estabishment of a minimum animal recording system. This case refers to Nepal, Azerbaijan, and Romania. Representatives of these countries have participated in various ICAR meetings, but also in this case no issue for involvement in ICAR has yet been found. Cooperative projects with ICAR member countries in order to establish a minimum recording system would be, I think, the only solution, and will be highly appreciated by the countries themselves. However, difficulties are many: from finding the appropriate local people who believe in the project and work for it, to finding the ICAR people who dedicate time and money to the project, to finally finding the sponsor.
There are two further areas where dairy buffalos will get more and more important in the future: China and South America. China has a huge variety of buffalo genetic resources, all of the swamp type, and provide a good deal of products to the farmers. In order to improve milk production, group of river type buffaloes (Murrah and Nili Ravi) were imported by the Buffalo Research Institute of Nanning, Guanxi to produce crossbred and triple-crossbred. The government has recently implemented a buffalo improvement project to increase milk production, which foresees further import of breeding stock and semen from top dairy breeds.
In several countries of South America (mainly Argentina, Brazil and Venezuela) dairy buffaloes were imported from Europe and India a few decades ago and there is an increasing research activity in various universities to improve buffalo productivity.

Fat content

The fourth aspect to be examined is the possible involvement of the laboratory WG in the activity of the mentioned countries.
It is therefore necessary to examine the statistics of milk recording activity with regard to laboratory activity. Fat content is important, therefore it is recorded in all countries where milk yield is recorded, but it is not as important as yield, therefore it is generally not recorded on all animals. An explanation to the little importance of fat in general is due to the fact that the more milk is produced, the more fat is also obtained; milk yield is more important and also less expensive to record. Therefore, in those cases, selection is done on milk yield mainly (Pakistan, India, Egypt).
However, let us have a look at the average fat percentage obtained in the milk of recorded buffaloes in the different countries in the table below

Country	no. recorded lactations	avg. fat %	st. dev
Azerbaijan	270	8.4	0.03
Italy	24,004	8.28	0.87
Egypt	249	8.0	1.95
Bulgaria	412	7.56	0.69
Brazil	306	7.04	1.36
India - Gujarat		6.68-7.01	(0.55-0.60)
Turkey	56	6.6	0.42
Iran	13,236	6.6	1.5

it is amazing to note the huge variability between butterfat content in the different countries. As researchers, we immediately would like to find the reason for such differences. Is it the breed? Is it the feeding system? Is it the analytical method? Unfortunately, poor interest in comparing milk recording results between countries arose after almost ten years of ICAR activities. Every country seems to be happy the way they are. The breed is of course very important. But in Azerbaijan, the same breed as in Iran is reared (Azari breed). Could the reason be inside the feeding system? Only in Italy (and maybe in Egypt in commercial farms) buffaloes are fed quite intensively respecting their nutritive energy and protein requirements. In most country they are fed by-products and allowed grazing whenever possible. I believe therefore that the analysis of fat content could be a good start for a cooperative multi-country laboratory project, because, provided that analysis results are reliable and comparable between different laboratories, many answers will be obtained as regards to genetic types and feeding practices.

Protein content

Protein content, as part of the milk recording activity and for selection purposes, is recorded only in Italy and Bulgaria. This fact is not amazing because only in Italy and Bulgaria cheese processing from buffalo milk is economically important. To be more correct, also in Egypt and Iran cheese making is important, but because the market does not request specific full buffalo milk cheeses (cheese is generally produced from mixing milk of various livestock) and because of the analysis costs, protein is just left behind.
Italy is a very peculiar case: all buffalo milk is processed into mozzarella cheese, which has a special stamp and a higher value if it is made from only buffalo milk. Therefore, protein content is not only necessary, but it is the main parameter taken into account in the bull genetic merit (GM), which is given by the following formula:
GM= kg milk * [(3.5 * (prot. %) + 1.23 * (fat %) – 0.88] /100.
In every country buffalo milk is paid more than cow's milk, but in no country the price difference is as huge as in Italy (from three to four times more). The importance of protein content is evident from the above algorithm used to calculate animal genetic merit. However, in the case of mozzarella cheese, and it might be true also for other buffalo dairy products around the world, fat content is extremely important, therefore, contrary to what is happening to many dairy cattle breeds, where selection against high fat content is being performed, high fat content in buffalo milk is requested and appreciated. Dairy experts refer that the high fat content in buffalo milk influences mozzarella cheese composition, fat loss and cheese texture, curd melting and stretching propterties (Addeo et al. 1996).
As the buffalo producers all over the world will become aware of the importance of buffalo milk quality for reaching international markets, both for the sale of milk and of breeding stock, the basic milk analyses of fat and protein that are already compulsory for any recording system in Europe will become a must.

Somatic Cell Count

Italy is the only country where somatic cell count (SCC) is routinely registered, i.e. at each monthly test. On the one side this is the consequence of the fact that buffalo recording system in Italy fully reflects the dairy cow system, therefore what is performed for cattle, is automatically performed for buffalo. SCC was indicated to be a good parameter to detect sub-clinical mastitis (Boichard and Rupp, 1997), therefore it was soon adopted as a compulsory parameter to be provided by the recording organizations. For the major dairy cattle breeds, a somatic cell count genetic merit is calculated and produced in Italy. SCC in buffalo milk has also become part of the output provided by the recording system but it is not yet used for bull selection.
It is common opinion in the Asian countries that buffalo do not suffer mastitis, but I have no statistics to show. I have no statistics either on mastitis in the other countries; the only existing results, limited to Italy, are referred to SCC data in five different herds (Moioli et al., 2003). In this study, 9,807 observations of test-day SCC of 1,785 calvings (1996 to 2002) of 757 buffaloes were used. Average SCC was 181,000 (S.E. 343,000) and was significantly affected by year and season, age at calving, stage of lactation and herd. In fact, the highest SCC levels were found in the year 2002, in the months of May, August and December, in the older buffaloes and at the end of lactation. The effects of the age of the cow and lactation stage showed the same trend as with dairy cows (Kennedy et al.,1982; Harmon, 1994): this means that as animals get older, SCC increases and that SCC are highest at the end of the lactation.
Herd effect was highly significant and it was the effect absorbing the highest amount of variability, i.e. the most important effect.
Moreover, we found that milk yield, fat and protein percentage were negatively affected by SCC. An increase by 100,000 in SCC produced a decrease of 0.85 kg in the daily milk yield; a decrease of 0.028 in fat percentage and of 0.043 in protein percentage. Similar results were obtained for dairy cows by Kennedy et al. (1982) and Tripaldi et al. (2003) that gave negative correlation coefficients between test-day SCC and milk yield, fat and protein percentage.
The range of values for SCC found in the present work (mean=281,000; S.E.=343,000) confirms that buffalo on averageare less affected by the presence of somatic cells in milk than the dairy cows (Ng-Kwai-Hang et al., 1984) and sheep (Haenlein et al., 1973), while agreeing with the values found for buffalo both in Italy (Tantillo et al., 1997) and in South America in buffaloes originating from Asia (Silva and Silva, 1994).
SCC is a good indicator to define individual health status of most livestock, but in particular of buffalo which is generally managed in worser sanitary conditions than dairy cattle. Such conditions badly affect milk yield to a stronger extent compared to what was observed by Kennedy et al. (1982) for dairy cattle.

Moreover, in an experimental herd where also analyses for pathogens are being performed, it was evident that mastitis in buffalo exists and it is increasing. The belief that buffalo does not suffer from mastitis should not only be rejected but programmes for a better knowledge of the extent of this disease also in the other countries should be considered. This would be a very interesting topic to approach for the Laboratory WG.

Conclusions

Possible involvement of the ICAR Laboratory Network might be directed to:

- ICAR member countries for harmonization of milk analysis procedures;
- Non-ICAR members with long tradition of buffalo recording for co-operative research projects;
- Countries with no tradition of buffalo recording but where buffaloes are economically important (Azerbaijan, Nepal, Romania) for co-operative development projects;
- Countries with an emerging importance of dairy buffalo (China, Brazil).

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Milk performance recording of buffalo

Azerbaijan Brazil Bulgaria

Egypt India

Greece Iran

Syria Italy

Nepal Pakistan

Syria Turkey United Kingdom

- **Italy**
- **Bulgaria**
- **Gujarat**
  - –ICAR members
  - –Long tradition buffalo recording

- Pakistan and Iran:

- well established milk recording systems and selection

- Nepal
- Azerbaijan
- Romania

contact people are ready to co-operative projects

China:
Nanning Research Institute

South America:
universities of
Argentina
Brazil
Venezuela

•What analyses?

Country	n.	Fat %	St dev
Azerbaijan	270	8.4	0.03
Italy	24,004	8.28	0.87
Egypt	249	8	1.95
Bulgaria	412	7.56	0.69
Brazil	306	7.04	1.36
Gujarat	3,500	6.68-7.01	0.55-0.60
Turkey	56	6.6	0.42
Iran	13,236	6.6	1.5

- Buffalo genetic merit in Italy

- **Kg milk**
- **Protein x 3.50**
- **Fat x 1.23**

- Somatic Cell Count is indicated as good parameter to detect sub-clinical mastitis

- Somatic cell count

- 9807 records
- 1785 lactations
- 757 buffaloes
- 5 farms

- Results

- 1. Herd effect
- 2. age
- 3 stage of lactation

Milk yield, fat and protein are negatively affected by SCC

- Conclusions

- Different realities can be targeted by the lab network
- - ICAR members: harmonisation
- - non-ICAR members with good systems
- - developing countries

Sheep, goat and buffalo milk analyses – Problems and difficulties with reference and routine methods

Luca Lattanzi, Ugo Paggi

A.I.A. Laboratorio Standard Latte, Via dell'Industria 24, Maccarese (Roma), Italy

Reference and routine methods have been defined specifically for cow milk analyses.Those methods are normally applied for other species too.
But precision figures r and R for these species are often higher than figures for cow milk: why?
In A.I.A.- Laboratorio Standard Latte some experiments have been carried out and are partly still underway in order to find out what the explanation is for these differences.

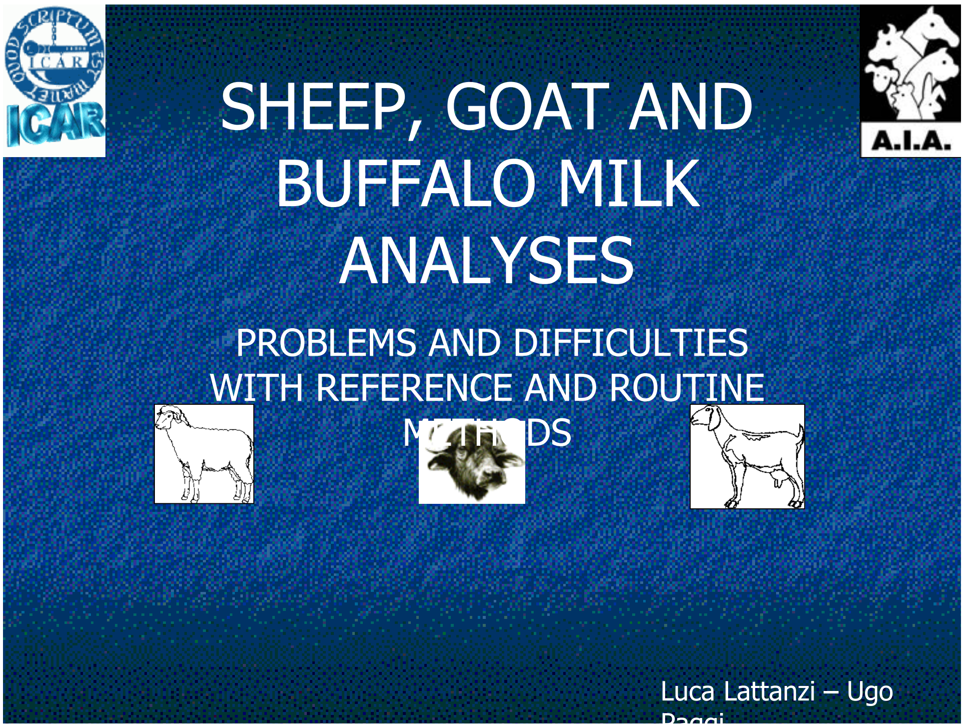
With regard to the Kjeldahl reference method for nitrogen/protein we need to focus on:

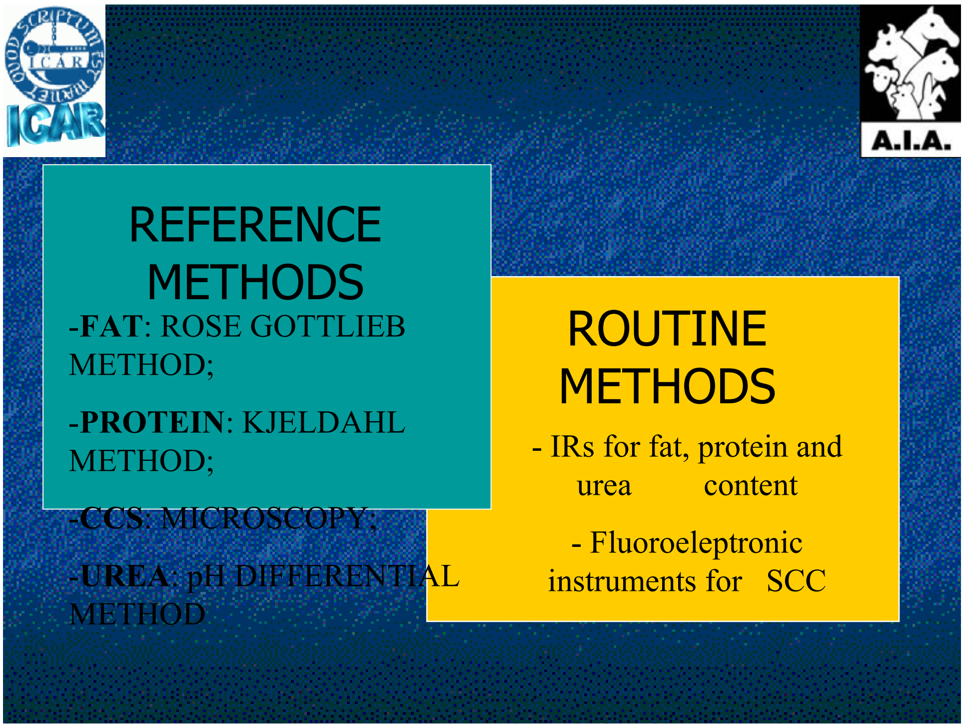
- defining the best quantity of milk to be analyzed in different milk types;
- standardizing the analytical procedures among labs (questionnaires sent in some proficiency test demonstrate that not all the labs apply strictly the ISO Standard);
- optimization of catalyst and sulfuric acid use in relationship to nitrogen and fat content.

About Rose Gottlieb reference method for fat we tried to find the differences (such as matrix) for the different species:

- Goat milk is characterized by a different fatty acid compositionWe can assume that fatty acids are to a lesser extent recovered by the reference method. Tests have been carried out adding known quantities of butyric acid (C4), esanoic acid (C6) and oleic acid (C18). Results show that butyric acid is not recovered at all while better recovery (C6) and full recovery (C18) was obtained in case of longer carbon chains. Next steps will be recovery experiments with different fatty acids recovery (to confirm the correlation between recovery and carbon chains length) and the optimization of the ammonia quantity.
- Buffalo and sheep milks are characterized by high content of fat, so the first supposition is that the amount of solvents applied may not be enough for milk of these species. But tests carried out (four extractions instead of three, addiction of extra ammonia during the second extraction) did not show any difference.

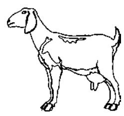
In routine analysis on fat, protein and urea content, infrared instruments are normally utilized: Also here standard deviations and precision figures were found less favourable than those for cow milk.Proficiency tests on sheep and goat milk carried out in November 2003 showed that even if figures halve after calibrations, they still remain unsatisfactory. Again, matrix effects could be the reason for these differences. In order to test the matrix effect, goat milk (the most similar in composition to cow milk) wasanalyzed on a MilkoScan FT120 with two different channels:
- a specific cow milk channel calibrated over the years with cow milk reference material;
- a specific channel for goat milk calibrated over the years with goat milk reference material.
Both channels were set starting with slope=1 and bias=0.
The goat milk analyzed gave acceptable response on the cow milk channel in fat content, while for protein content the differences were higher than expected (mean of differences of 0,20 g/100g).
As usual, conclusions are not easy to draw, but we can confirm that matrix effect subsists for both reference and routine methods: new tests should be carried out and standards optimized and adapted to each species.


SHEEP, GOAT AND BUFFALO MILK ANALYSES
PROBLEMS AND DIFFICULTIES
WITH REFERENCE AND ROUTINE
Luca Lattanzi – Ugo
A.I.A.
ICAR


REFERENCE METHODS
-FAT: ROSE GOTTLIEB METHOD;
-PROTEIN: KJELDAHL METHOD;
-CCS: MICROSCOPY;
-UREA: pH DIFFERENTIAL METHOD
ROUTINE METHODS
- IRs for fat, protein and urea content
- Fluoroeleptronic instruments for SCC
A.I.A.
ICAR

ICAR INTERLABORATORY PT ON GOAT MILK NOVEMBER 2003

PARTICIPANT LABORATORIES (in alphabetical order)
INTERLABORATORY PROFICIENCY STUDY ON GOAT MILK NOVEMBER 2003



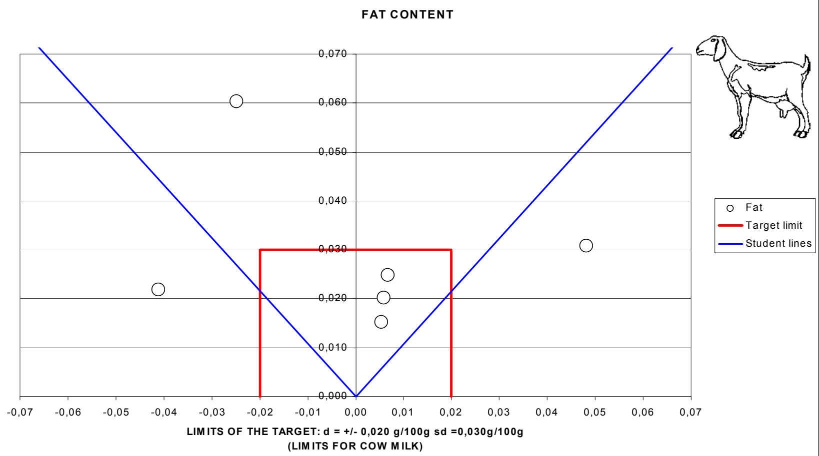
AIA LABORATORIO STANDARD LATTE - ITALY
C.M.I.O.- CYPRUS
CENTRAL MILK LAB ICBA - ISRAEL
FEDERAL DAIRY RESEARCH STATION - SWITZERLAND
LABORATORIO AGROALIMENTARIO DE SANTANDER - SPAIN
TINE MEIERIET OST BRUMDDAL - NORWAY
UNIVERSITY OF LJUBLIANA, BIOTECHNICAL FACULTY - SLOVENIA

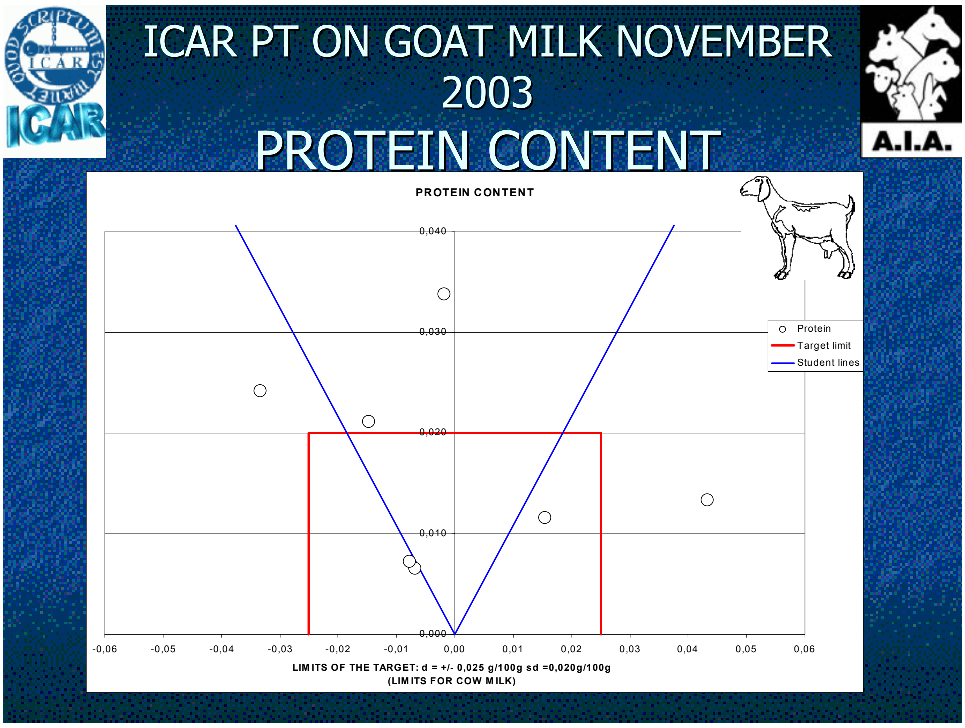
LAB CODE	FAT*	PROTEIN*	LACTOSE	SOMATIC CELLS
1		Kjeldahl		Fossomatic 5000
2	Rose-Gottlieb	Kjeldahl	Enzymatic	Not specified
3	Rose-Gottlieb	Kjeldahl	Diff. pH	Fossomatic 5500
4		Kjeldahl	CL-10	Fossomatic 400
5	Rose-Gottlieb	Kjeldahl	Not specified	
6	Rose-Gottlieb	Kjeldahl	Enzymatic	Fossomatic 5000
7	Rose-Gottlieb	Kjeldahl		Somacount 150

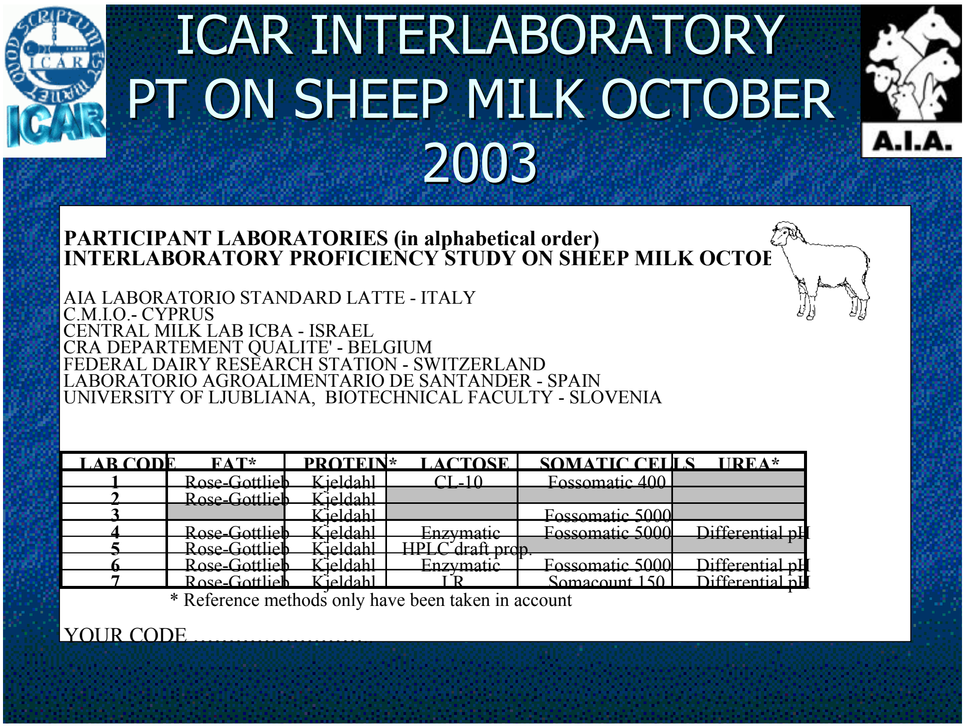
* Reference methods only have been taken in account

ICAR PT ON GOAT MILK NOVEMBER 2003

FAT CONTENT







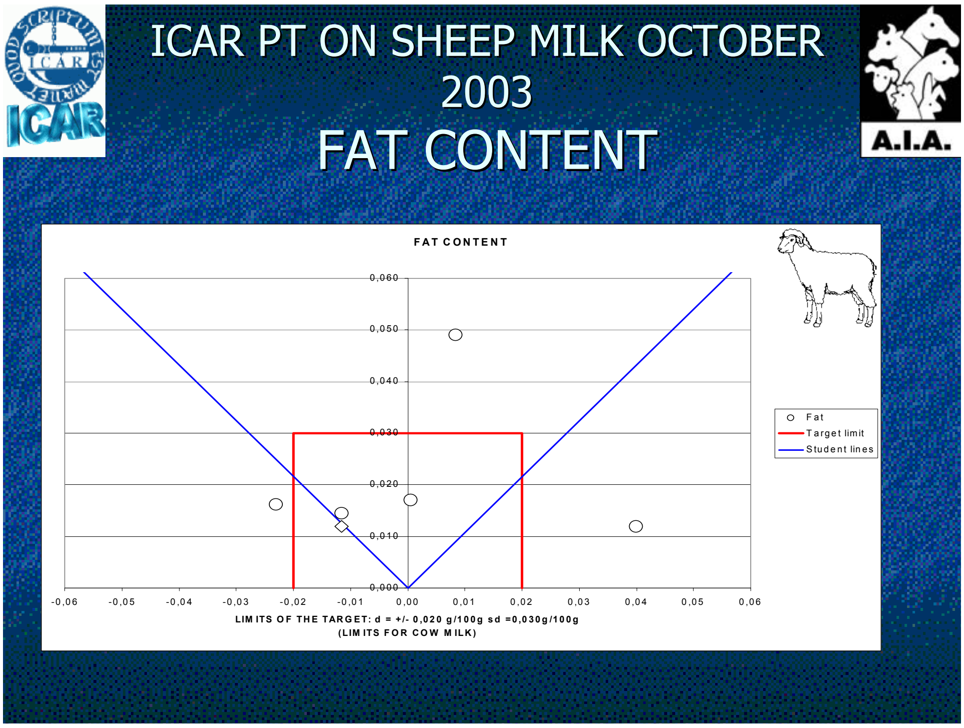
PARTICIPANT LABORATORIES (in alphabetical order)
INTERLABORATORY PROFICIENCY STUDY ON SHEEP MILK OCTOB

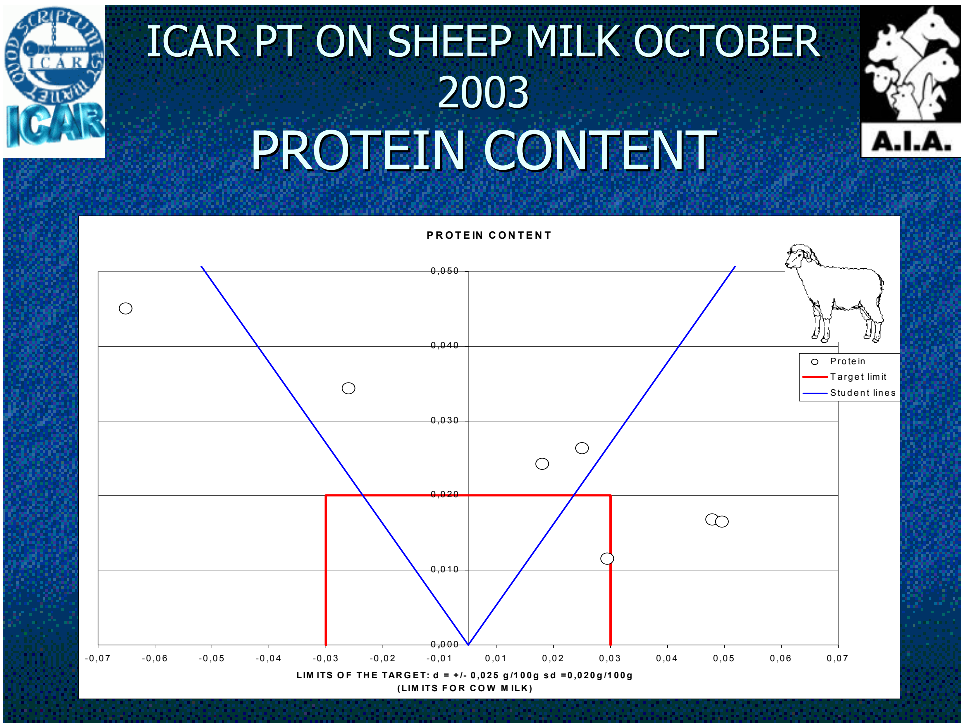
AIA LABORATORIO STANDARD LATTE - ITALY
C.M.I.O.- CYPRUS
CENTRAL MILK LAB ICBA - ISRAEL
CRA DEPARTEMENT QUALITE' - BELGIUM
FEDERAL DAIRY RESEARCH STATION - SWITZERLAND
LABORATORIO AGROALIMENTARIO DE SANTANDER - SPAIN
UNIVERSITY OF LJUBLIANA, BIOTECHNICAL FACULTY - SLOVENIA

LAB CODE	FAT*	PROTEIN*	LACTOSE	SOMATIC CELLS	UREA*
1	Rose-Gottlieb	Kjeldahl	CL-10	Fossomatic 400	
2	Rose-Gottlieb	Kjeldahl			
3		Kjeldahl		Fossomatic 5000	
4	Rose-Gottlieb	Kjeldahl	Enzymatic	Fossomatic 5000	Differential pH
5	Rose-Gottlieb	Kjeldahl	HPLC draft prop.		
6	Rose-Gottlieb	Kjeldahl	Enzymatic	Fossomatic 5000	Differential pH
7	Rose-Gottlieb	Kjeldahl	LR	Somacount 150	Differential pH

* Reference methods only have been taken in account

YOUR CODE ....


ICAR PT ON SHEEP MILK OCTOBER 2003
FAT CONTENT
ICAR
A.I.A.
FAT CONTENT
0,060
0,050
0,040
0,030
0,020
0,010
0,000
-0,06 -0,05 -0,04 -0,03 -0,02 -0,01 0,00 0,01 0,02 0,03 0,04 0,05 0,06
LIMITS OF THE TARGET: d = +/- 0,020 g/100g sd =0,030g/100g
(LIMITS FOR COW MILK)
Fat
Target limit
Student lines


ICAR PT ON SHEEP MILK OCTOBER 2003
PROTEIN CONTENT
ICAR
A.I.A.
PROTEIN CONTENT
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0,040
0,030
0,020
0,010
0,000
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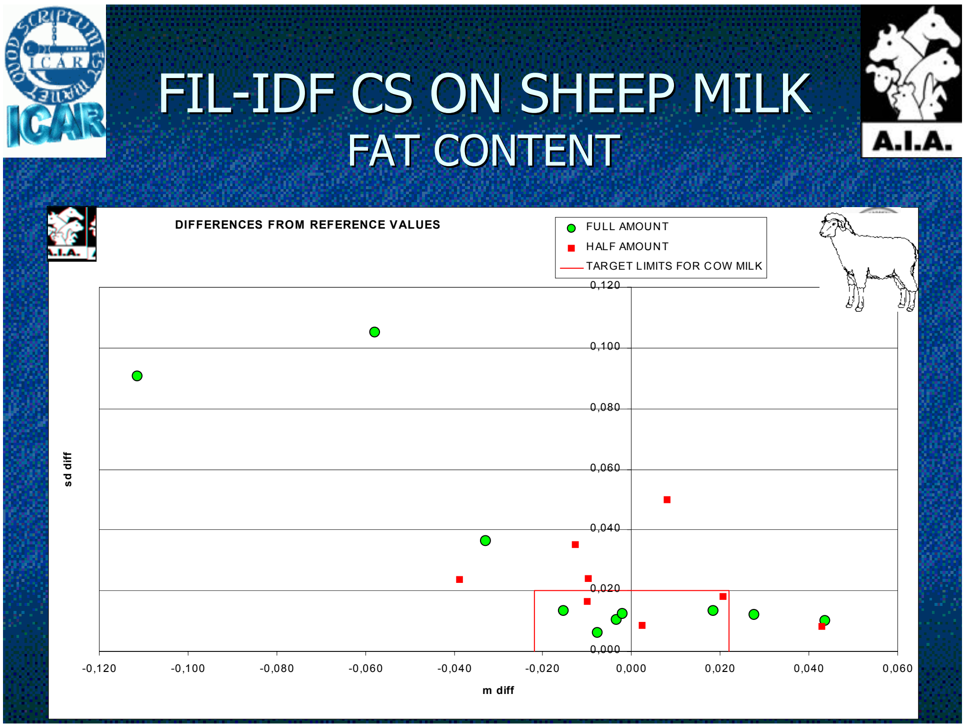
(LIMITS FOR COW MILK)
Protein
Target limit
Student lines


FIL-IDF CS ON SHEEP MILK
FAT CONTENT
DIFFERENCES FROM REFERENCE VALUES
FULL AMOUNT
HALF AMOUNT
TARGET LIMITS FOR COW MILK
0,120
0,100
0,080
0,060
0,040
0,020
0,000
sd diff
-0,120
-0,100
-0,080
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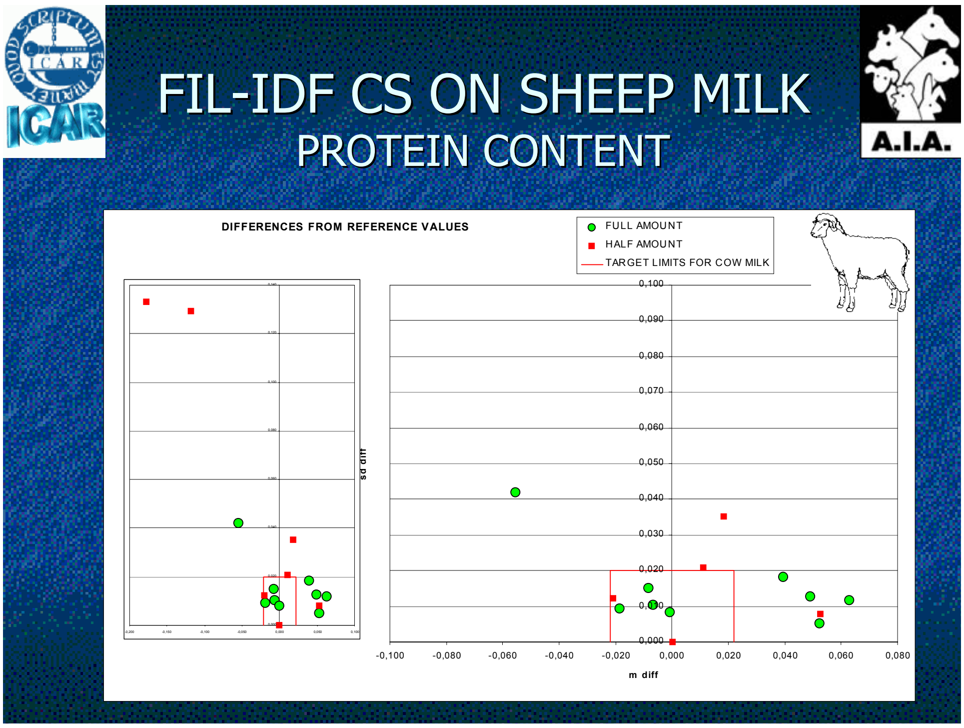
0,000
0,020
0,040
0,060
m diff
A.I.A.
ICAR


FIL-IDF CS ON SHEEP MILK
PROTEIN CONTENT
DIFFERENCES FROM REFERENCE VALUES
FULL AMOUNT
HALF AMOUNT
TARGET LIMITS FOR COW MILK
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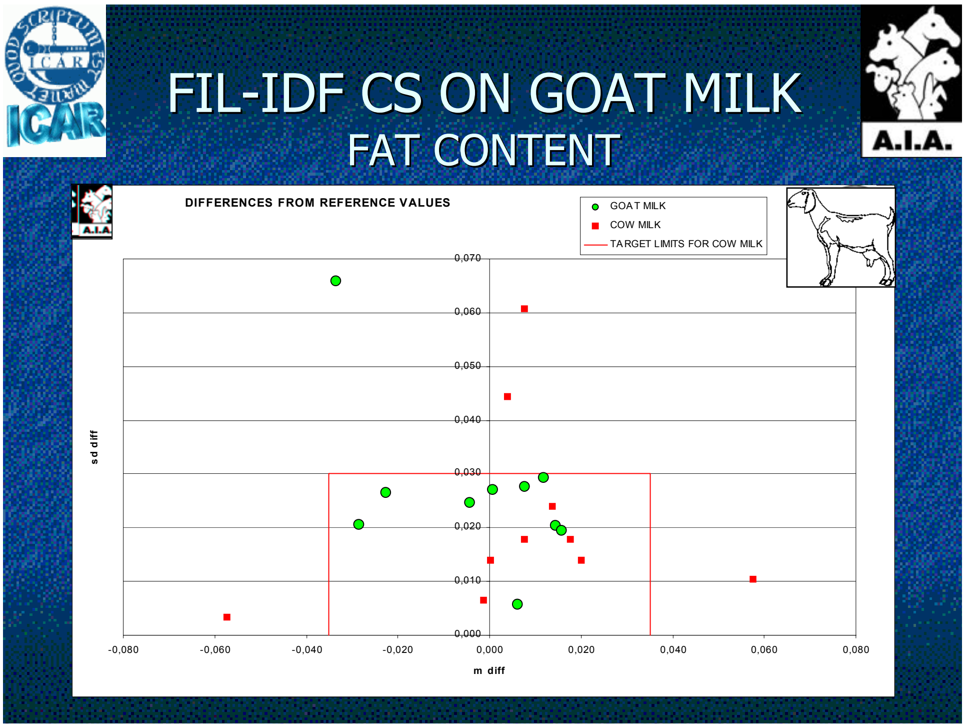
0,080
0,070
0,060
0,050
0,040
0,030
0,020
0,000
sd diff
-0,100
-0,080
-0,060
-0,040
-0,020
0,000
0,020
0,040
0,060
0,080
m diff
A.I.A.
ICAR


ICAR
A.I.A.
FIL-IDF CS ON GOAT MILK
FAT CONTENT
DIFFERENCES FROM REFERENCE VALUES
GOAT MILK
COW MILK
TARGET LIMITS FOR COW MILK
0,070
0,060
0,050
0,040
0,030
0,020
0,010
0,000
sd diff
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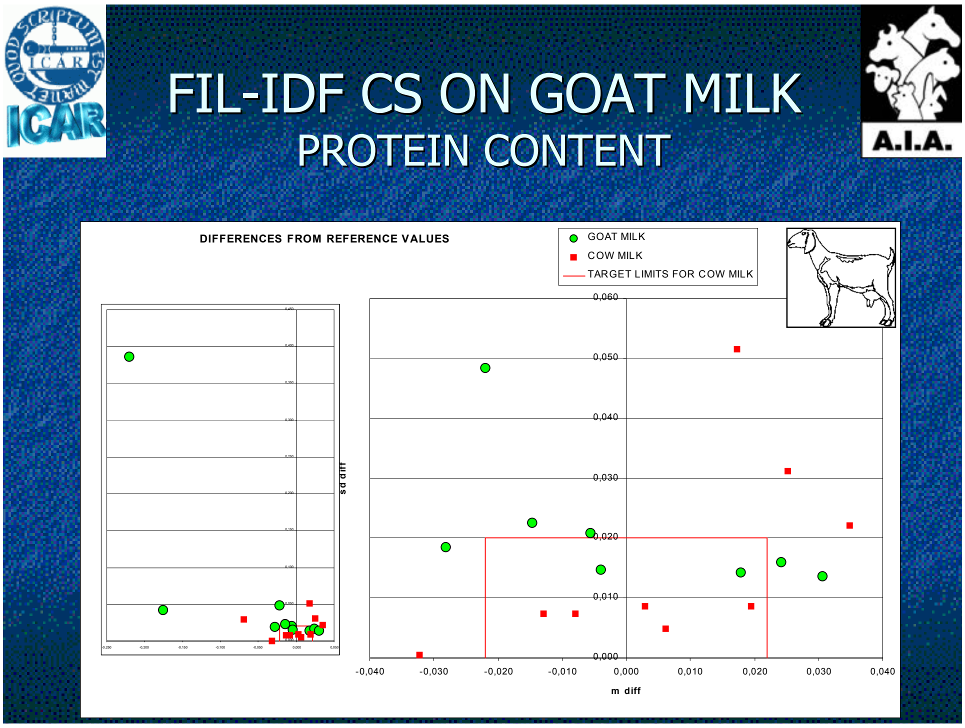
0,060
0,080
m diff


ICAR
A.I.A.
FIL-IDF CS ON GOAT MILK
PROTEIN CONTENT
DIFFERENCES FROM REFERENCE VALUES
GOAT MILK
COW MILK
TARGET LIMITS FOR COW MILK
0,060
0,050
0,040
0,030
0,020
0,010
0,000
sd diff
-0,040
-0,030
-0,020
-0,010
0,000
0,010
0,020
0,030
0,040
m diff

Kjeldahl repeatability among different species (LSL 2004)

	N° OF SAMPLES	Sr	r
COW	63	0,0162	0,05
GOAT	21	0,0206	0,06
SHEEP	48	0,0330	0,09
BUFFALO	25	0,0340	0,10

Kjeldahl Reference Method

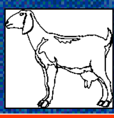
Points to focus on:

- To define the best quantity of milk to be analysed in different milk types;
- Standardization of analyses' procedures among labs;
- Reagents: quantity optimization of catalyst and sulfuric acid in relationship to Nitrogen and Fat content;

Rose Gottlieb repeatability among different species
LSL 2004

	N° OF SAMPLES	Sr	r
COW	66	0,0100	0,03
GOAT	21	0,0164	0,05
SHEEP	49	0,0161	0,05
BUFFALO	23	0,0165	0,05

Rose Gottlieb problems Goat milk

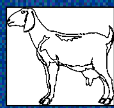


- Lypolisys

Test trial adding known quantities of FFA to milk

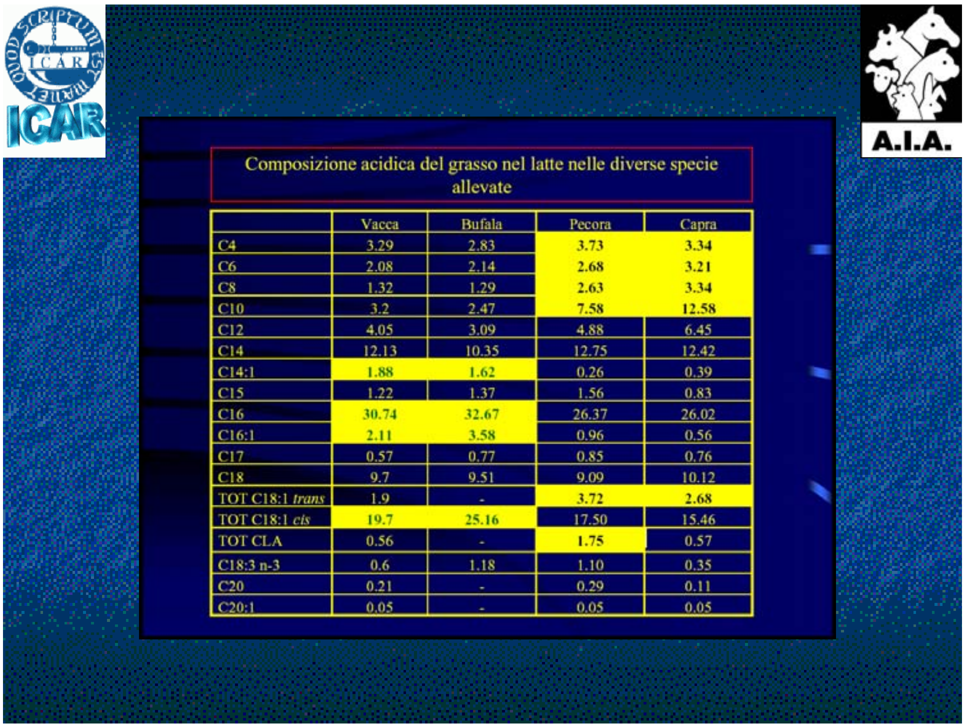
FFA Recovery Test
(LSL 2004)

- Cow milk as test sample: defined limits for repeatability and reproducibility
- Samples prepared adding known quantities of:
- Butirric Acid (C4)
- Esanoic Acid (C6)
- Olive oil (98% of fatty acids, with 70-80% of oleic acid C18)



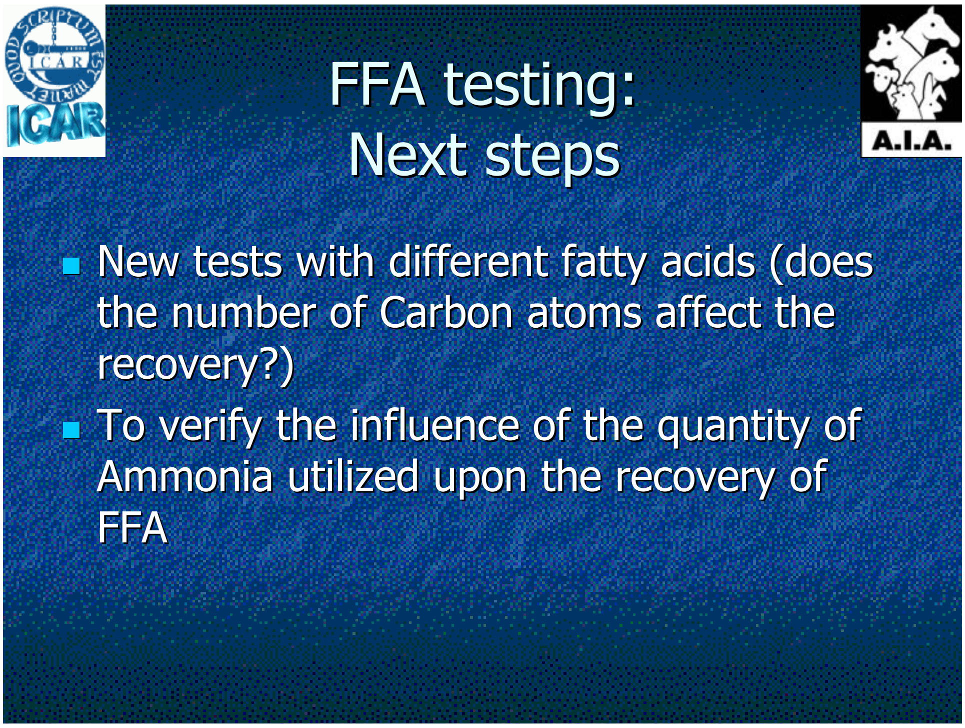
FFA recovery trial

	ADD.	1 DUPPLICATE	2 DUPLICATE	MEAN	EXP	EXP-MEAN
BASE MILK		3,54	3,53	3,54		
BUTIRRIC ACID	0,0464	3,53	3,52	3,53	3,58	0,06
BUTIRRIC ACID	0,0972	3,53	3,52	3,53	3,63	0,11
ESANOIC ACID	0,0524	3,52	3,53	3,53	3,59	0,06
ESANOIC ACID	0,1043	3,56	3,57	3,57	3,64	0,07
OLIVE OIL	0,1930	3,75		3,75	3,73	-0,02
OLIVE OIL	0,1300	3,66		3,66	3,67	0,01



Composizione acidica del grasso nel latte nelle diverse specie allevate

	Vacca	Bufala	Pecora	Capra
C4	3.29	2.83	3.73	3.34
C6	2.08	2.14	2.68	3.21
C8	1.32	1.29	2.63	3.34
C10	3.2	2.47	7.58	12.58
C12	4.05	3.09	4.88	6.45
C14	12.13	10.35	12.75	12.42
C14:1	1.88	1.62	0.26	0.39
C15	1.22	1.37	1.56	0.83
C16	30.74	32.67	26.37	26.02
C16:1	2.11	3.58	0.96	0.56
C17	0.57	0.77	0.85	0.76
C18	9.7	9.51	9.09	10.12
TOT C18:1 *trans*	1.9	-	3.72	2.68
TOT C18:1 *cis*	19.7	25.16	17.50	15.46
TOT CLA	0.56	-	1.75	0.57
C18:3 n-3	0.6	1.18	1.10	0.35
C20	0.21	-	0.29	0.11
C20:1	0.05	-	0.05	0.05



Rose Gottlieb problems Sheep and Buffalo milk

- High content of fat

- Are solvents enough?

LSL 2002 SHEEP MILK

FAT CONTENT

EXTRACTION OF FAT (g/100g) IN DIFFERENT VESSELS

EXTRACTION	I	II	III	IV
FIRST SAMPLE	8,165	0,230	0,138	0,0069
SECOND SAMPLE	7,470	0,267	0,0189	0,0030

LSL 2004
BUFFALO MILK

FAT CONTENT

EXTRACTION OF FAT (g/100g) IN DIFFERENT VESSELS

SAMPLE A		
SEPARATE EXTRACTIONS		
	RIP1	RIP2
1 ESTR.	9,462	9,713
2 ESTR.	0,4	0,175
3 ESTR.	0,011	0,017
4 ESTR.	0,004	0,007
TOT	9,877	9,912
MEAN	**9,895**	

SAMPLE B		
SEPARATE EXTRACTIONS		
	RIP1	RIP2
1 ESTR.	8,367	8,281
2 ESTR.	0,115	0,169
3 ESTR.	0,027	0,016
4 ESTR.	-0,001	-0,006
TOT	8,508	8,46
MEAN	**8,484**	

LSL 2002
SHEEP MILK

FAT CONTENT

COMPARISON BETWEEN STANDARD EXTRACTION AND ADDICTION OF 2ml EXTRA OF NH3 DURING SECOND EXTRACTION

N samples	2ml NH3	[illegible]
1	[illegible]	7,79
2	8,30	8,32
3	5,81	5,81
4	5,06	5,06

LSL 2004
BUFFALO MILK
FAT CONTENT

COMPARISON BETWEEN STANDARD EXTRACTION AND ADDICTION OF EXTRA 2ml OF NH3 DURING

	2ml NH3	4ml NH3
A	9,89	9,89
B	8,48	8,44
C	7,49	7,52
D	6,91	6,91

Routine methods:

Infrared Instruments

for fat and protein content

Protein content: standard deviation (before calibration) among different species

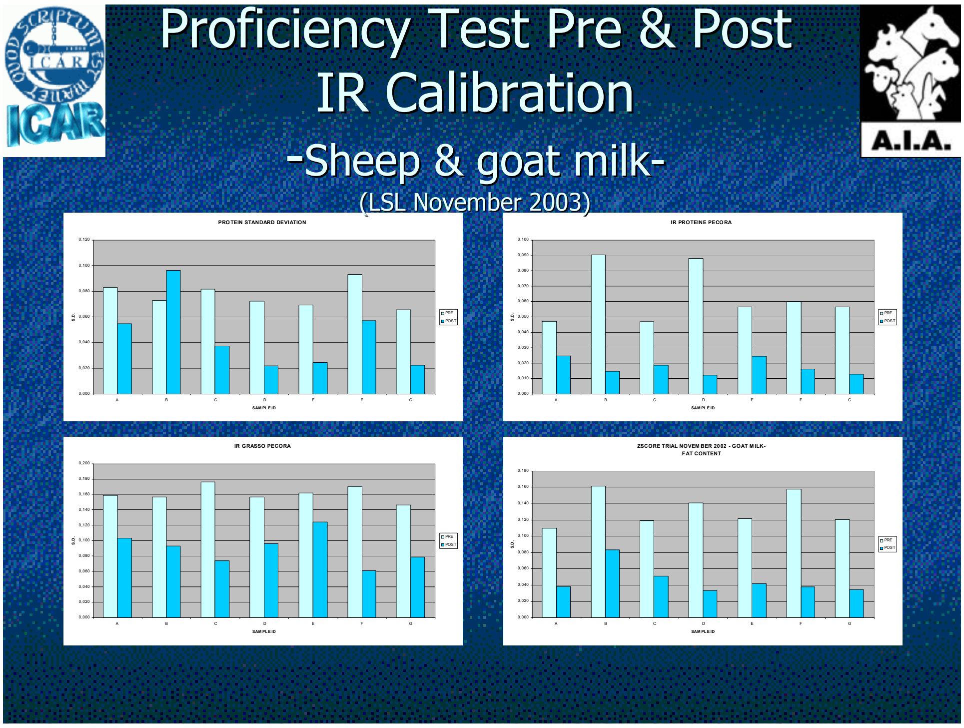
(LSL Jan-Feb. 2004)

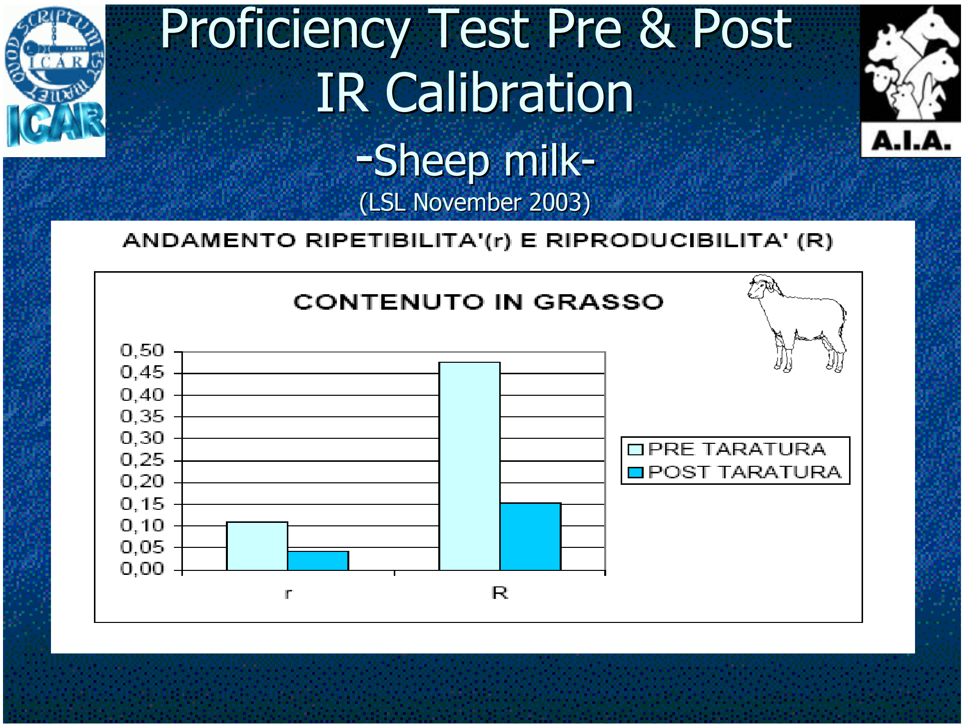
cow			goat			sheep			bufala		
lab #	MEAN	s.d.	lab #	MEAN	s.d.	lab #	MEAN	s.d.	lab #	MEAN	s.d.
23	2,43	0,036	16	2,59	0,043	14	4,18	0,094	11	3,84	0,048
23	2,84	0,029	16	2,61	0,105	14	4,61	0,048	11	4,16	0,018
23	2,92	0,031	16	2,95	0,069	14	4,68	0,032	11	4,17	0,029
23	2,95	0,029	16	2,97	0,059	14	5,04	0,020	11	4,21	0,061
23	3,00	0,031	16	3,29	0,100	14	5,16	0,016	11	4,47	0,053
23	3,04	0,020	16	3,38	0,047	14	5,40	0,051	11	4,54	0,077
23	3,06	0,032	16	3,64	0,039	14	5,70	0,025	11	4,64	0,090
23	3,24	0,025	16	3,82	0,057	14	5,78	0,050	11	4,73	0,052
23	3,24	0,028	16	3,88	0,023	14	5,89	0,301	11	4,82	0,112
23	3,31	0,036	16	4,04	0,037	14	5,98	0,032	11	4,87	0,031
23	3,47	0,029	16	4,33	0,046	14	6,24	0,033	11	5,02	0,059
23	4,06	0,025	16	4,85	0,057	14	6,40	0,086	11	5,45	0,124
Mean	3,13	0,030	Mean	3,53	0,062	Mean	5,42	0,099	Mean	4,58	0,070

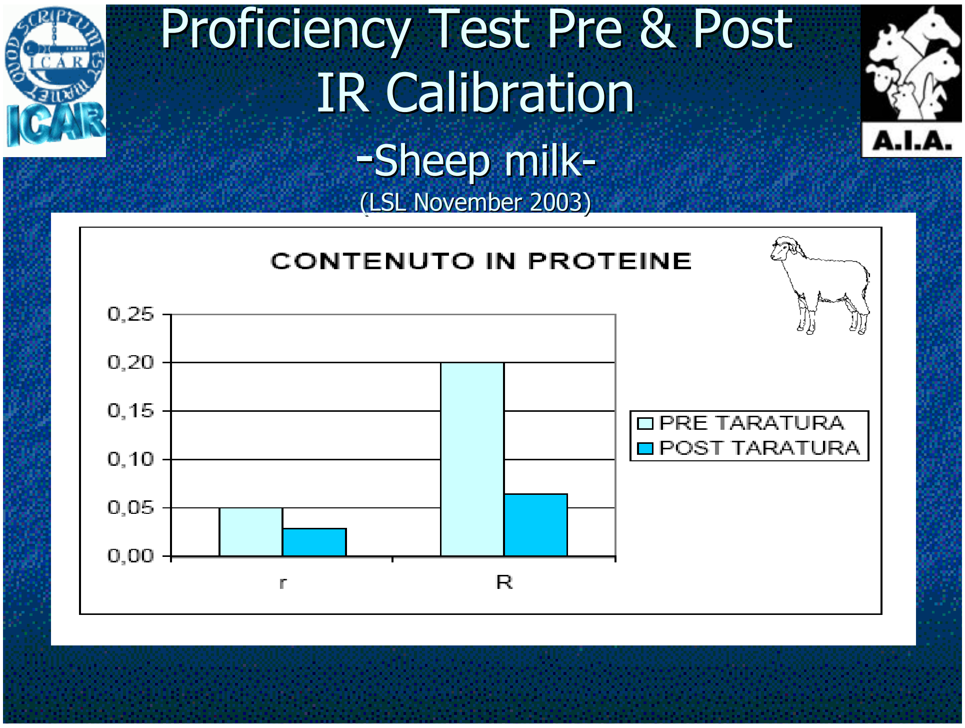
Fat content: standard deviation (before calibration) among different species

(LSL Jan-Feb. 2004)

cow			goat			sheep			bufala		
lab #	MEAN	s.d.	lab #	MEAN	s.d.	lab #	MEAN	s.d.	lab #	MEAN	s.d.
23	2,96	0,038	16	2,97	0,054	14	5,00	0,089	11	6,41	0,100
23	2,96	0,038	16	3,28	0,045	14	5,10	0,060	11	7,34	0,089
23	3,21	0,033	16	3,40	0,048	14	5,72	0,168	11	7,47	0,128
23	3,33	0,035	16	4,01	0,061	14	5,87	0,081	11	7,70	0,052
23	3,34	0,031	16	4,14	0,055	14	6,49	0,147	11	8,00	0,124
23	3,43	0,035	16	4,69	0,075	14	6,79	0,073	11	8,62	0,317
23	3,57	0,038	16	4,78	0,076	14	7,42	0,154	11	9,00	0,480
23	3,73	0,039	16	5,08	0,138	14	7,70	0,147	11	9,23	0,468
23	3,82	0,036	16	5,48	0,060	14	7,89	0,105	11	9,26	0,129
23	4,29	0,039	16	5,56	0,085	14	7,90	0,125	11	9,41	0,236
23	4,34	0,028	16	6,12	0,104	14	8,06	0,104	11	10,02	0,112
23	4,77	0,036	16	6,33	0,127	14	9,05	0,345	11	11,08	0,219
Mean	3,65	0,036	Mean	4,65	0,083	Mean	6,92	0,151	Mean	8,63	0,247


Proficiency Test Pre & Post
IR Calibration
-Sheep & goat milk-
(LSL November 2003)
PROTEIN STANDARD DEVIATION
IR PROTEINE PECORA
IR GRASSO PECORA
ZSCORE TRIAL NOVEMBER 2002 - GOAT MILK-
FAT CONTENT
A.I.A.


Proficiency Test Pre & Post
IR Calibration
-Sheep milk-
(LSL November 2003)
ANDAMENTO RIPETIBILITA'(r) E RIPRODUCIBILITA' (R)
CONTENUTO IN GRASSO
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
r
R
PRE TARATURA
POST TARATURA
A.I.A.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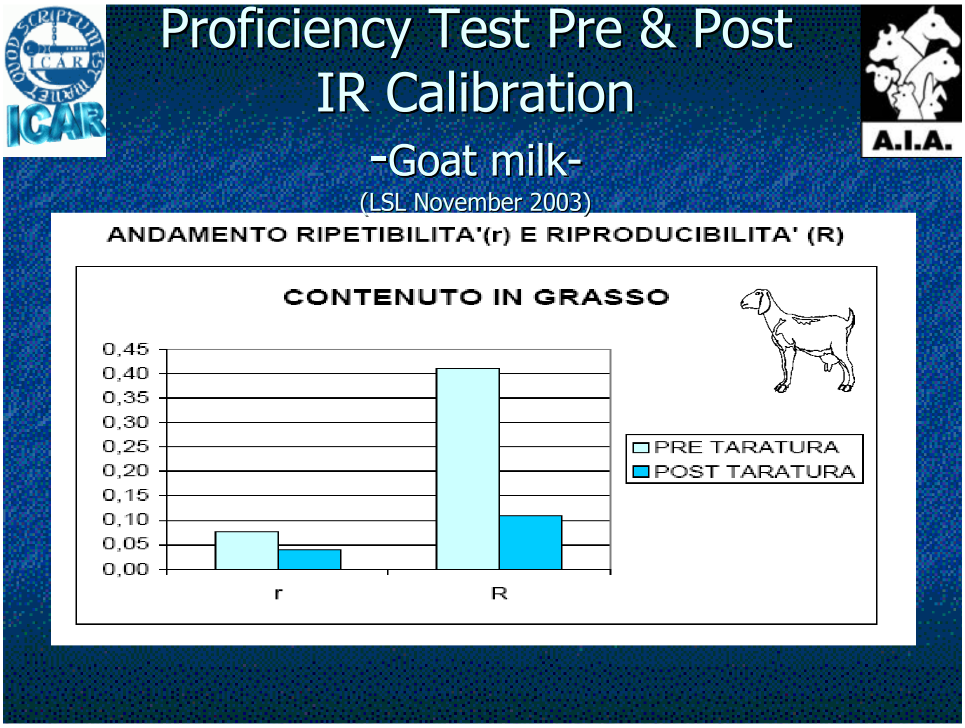
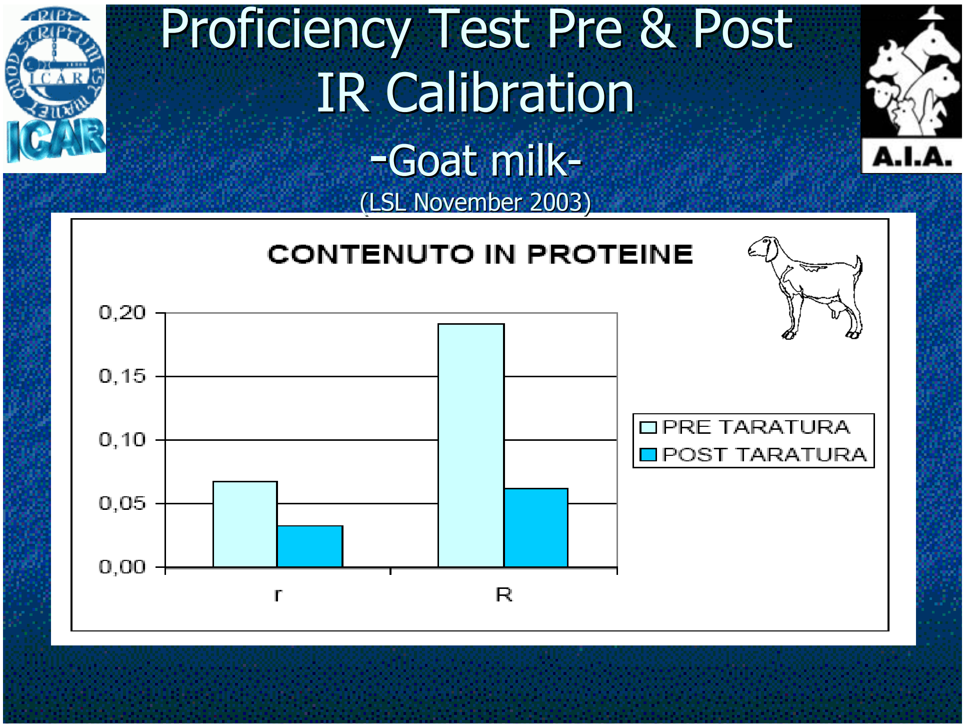
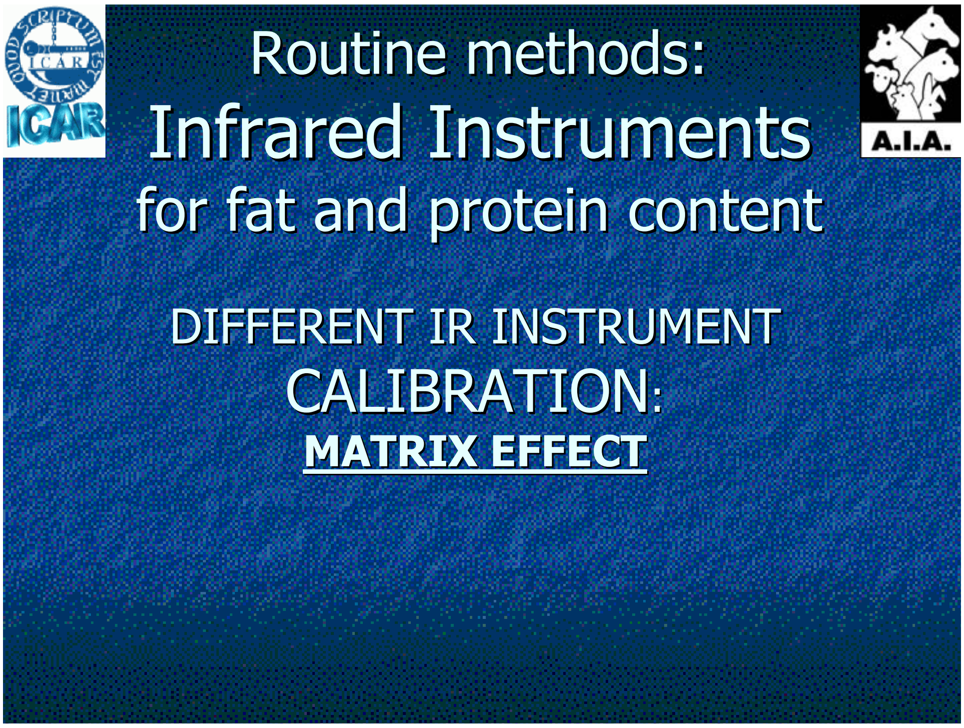
IR Calibration
-Sheep milk-
(LSL November 2003)
CONTENUTO IN PROTEINE
0,25
0,20
0,15
0,10
0,05
0,00
r
R
PRE TARATURA
POST TARATURA
A.I.A.
ICAR


Proficiency Test Pre & Post
IR Calibration
-Goat milk-
(LSL November 2003)
ANDAMENTO RIPETIBILITA'(r) E RIPRODUCIBILITA' (R)
CONTENUTO IN GRASSO
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
r
R
PRE TARATURA
POST TARATURA
A.I.A.
ICAR


ICAR
Proficiency Test Pre & Post
IR Calibration
-Goat milk-
(LSL November 2003)
A.I.A.
CONTENUTO IN PROTEINE
0,20
0,15
0,10
0,05
0,00
r
R
PRE TARATURA
POST TARATURA


ICAR
Routine methods:
Infrared Instruments
for fat and protein content
DIFFERENT IR INSTRUMENT
CALIBRATION:
MATRIX EFFECT
A.I.A.

DIFFERENT IR INSTRUMENT CALIBRATION: GOAT MILK ANALYSED ON TWO DIFFERENT CHANNELS

(LSL 2004 INSTRUMENT: FT120)

	FAT		PROTEIN		LACTOSE	
SAMPLE ID	GOAT CH.	COW CH.	GOAT CH.	COW CH.	GOAT CH.	COW CH.
1	4,10	4,07	3,21	3,04	4,60	4,58
2	2,99	3,00	3,56	3,93	4,70	4,67
3	2,87	2,78	2,57	2,42	4,66	4,64
4	3,56	3,54	3,13	2,95	4,57	4,53
5	5,62	5,57	3,19	3,05	4,55	4,52

CONCLUSIONS

AS USUAL , CONCLUSIONS ARE NOT EASY TO DRAW:

- Matrix effect sussists for both reference and routine methods;
- Amounts of test sample and reagents utilised should be optimezed for the different species;
- Need of maximum collaboration between labs in order to validate new modifications to the reference methods

Harmonised protocol for the evaluation of milk analysers and their international approval for milk recording

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Summary

Routine raw milk analysis plays a basic role in all milk payment and DHI schemes. Milk analysers as partially or fully automated systems are widely used in routine milk testing. With the "protocol for the evaluation of milk analysers" ICAR sets a standard for approval of milk analysers on an international scale, which is unique and prospective for the benefit of all users of raw milk analysers in the dairy world. ICAR approval of raw milk analysers will bring safety, confidence and cost reduction for users as well as for producers of milk analysers.

ICAR approval sets a standard of minimum requirements. In this paper the objectives of the protocol are described, the technical content is outlined, mentioning the rules of approval, the technical assessment and the demands for reports. The two annexes to the protocol give comprehensive assistance in doing the statistics and going through an approval procedure exemplarily.

Keywords: evaluation of milk analysers, international approval for milk recording, ICAR approval, analytical quality assurance, routine methods, DHI-schemes;

Introduction

Milk analysers as partially or fully automated systems are widely used in routine milk testing. There is broad application of different methods, different types of analysers and different technical solutions in various regulatory, organisational and operational environments. Data obtained with milk analysers are crucial for DHI schemes, for farm management and for the calculation of breeding values etc.

Being so important for the dairy world, there are high demands for precision and reliability of data produced with raw milk analysers. As a consequence there are various and different systems for approval of such analysers, depending on the country, national and regional regulations, the philosophy of the respective DHI and breeding organisations and many other factors being of influence.

When a new analytical instrument for raw milk analysis appeared on the market in the past, almost every new user did some work on the new analyser to get an impression of its function, its capabilities and its precision data. In total, there was much identical work done again and again and companies as well as new users spent much money for approval procedures on different levels, which had to be refinanced by the price for the analysers on the market or by charging for the analysis of samples. On the other hand, not every testing lab has the expertise to perform a fully expressive evaluation procedure. Some failures happened and there were different informations on the same analyser obtained by different investigators, which caused troubles and insecurity for interested people trying to get an overview of the evaluation reports for a certain device.

Because correct functioning and precision of analysers have a huge influence on safety and comparability of data in DHI schemes, breeding and farm management, ICAR declared its high interest in harmonizing the procedure of approval and setting an international standard.

The "protocol for the evaluation of milk analysers for ICAR approval" is the result of this intention of ICAR and derives from profound work of the Working Group on Milk Testing Laboratories (WG MTL), which sets the technical standard for an internationally recognized approval of milk analysers for milk recording.

The objectives of the protocol are

to provide the technical procedures to apply in order to get sufficient and comparable information on instrument suitability for milk recording use to ICAR countries and/or ICAR organisations; this should enhance confidence in analytical characteristics measured;
to provide the basis for an ICAR approval procedure; with an ICAR approval there is no need for further evaluation for organisations willing to use the analyser; this should reduce costs and labour for users significantly.

ICAR approval sets a standard of minimum requirements. This does not prevent those organisations, that are more demanding with respect to the evaluation of new analytical methods and tools, to go more into details than ICAR approval does. On the contrary, such additional work could enlarge the database for the respective analyser and support the efforts of ICAR to provide an optimum of information to the users continuously.

Technical basics – Content of the Protocol

The aim of the document is to define an overall procedure from the request for the approval to the procedure of the approval, the description of the technical evaluation and the procedure for the decision concluding on the approval request.
The document complies with ISO standard 8196 (IDF 128) and applies for the relevant parameters fat content, protein content, lactose content, somatic cell count (SCC) and urea content within the species cows, goats, ewes and buffaloes.

Rules of approval

Evaluation is done on four principal stages:

- general technical evaluation by an expert lab experienced in analytical evaluations as well as competent in the respective reference methods;
- test under routine conditions in at least two milk recording laboratories for at least two months;
- national approval can be obtained by asking a national competent body for nomination of the labs to do the evaluation reports mentioned above; an expert committee examines the reports and decides on a national approval;
- international approval of ICAR can be sought by doing the national approval procedure in three different countries; taking into account the technical evaluation report of WG MTL, ICAR Board will finally decide on the international approval.

Course of operations of a technical evaluation

Based on a principal explanation of the signal treatment in analysers, the different sources of variation and failures are explained and the basic criteria for the evaluation of proper functioning of an analyser are deducted.
The technical evaluation is based upon checking the minimum requirements an analyser has to fulfil. These are repeatability and short-term stability, carry-over effects, linearity and upper and lower measurement limits as basic instrumental fittings. The overall accuracy is evaluated by testing repeatability and accuracy of the mean including the exactness of calibration. In addition some more informative investigations are described to paint a comprehensive picture of the capabilities of an analyser due to the requirements of a DHI laboratory. These investigations are dealing with ruggedness, taking into account the interactions of the major milk components as well as biochemical changes in components due to lipolysis, protelysis and lactic souring e.g. The sample history plays an important role as well for the quality of analytical data; thus the effects of preservatives, of milk intake temperature and storage conditions are checked.
Besides those properties assessing ruggedness, practical issues are to be reported like speed of analysis, robustness of the instrument, monitoring facilities and servicing and the validation of precision under routine conditions.
In all parts of the protocol emphasis was laid on the task to provide clear description of all steps and calculations and to illustrate how to do the evaluation process in practise. From the preparation of test milk samples and the clear instruction how to do the measurement to the calculation of statistics and the interpretation of results all steps are described extensively.

Report and approval delivery

Reports have to follow the structure of the protocol and must be delivered by the organisation seeking for approval to the ICAR secretariat, including all informations on the evaluation process, results of analytical performance, discussion and comments or summary. Analytical raw data have to be provided electronically to enable further statistical evaluation.

Annexes

Two annexes clarify and illustrate all necessary statistical formulas for method evaluations (annex A) and give examples of calculation and presentation of evaluation results using real data sets (annex B).

Conclusion

Results of routine milk testing have an enormous economical, practical and scientific impact on animal breeding and husbandry in dairy livestock. With the protocol for the evaluation of milk analysers ICAR sets a standard for approval of milk analysers on an international scale, which is unique and prospective for the benefit of all users of raw milk analysers in the dairy world. ICAR approval of raw milk analysers will bring safety, confidence and cost reduction for users as well as for producers of milk analysers. During the next months ICAR will fix the procedure for application. Then it is up to the users and producers to draw the benefit out of it.

Meeting of ICAR Reference Laboratory Network
31st May 2004 – Sousse, Tunisia

Protocol For The Evaluation Of Milk Analysers For ICAR Approval

Christian Baumgartner
on behalf of WG MTL

Meeting of ICAR Reference Laboratory Network

Agenda

- introduction and general intent of the paper
- structure of the paper
- technical content
- procedure of application
- other species than cow

Introduction

- Routine raw milk analysis plays a basic role in all milk payment and DHI schemes.

	Bavaria	Germany	World*
labs	1	19	800 (2.000)
samples/ year	15 Mio.	40 Mio.	300 – 500 Mio.
analyses	54 Mio.	140 Mio.	1.000 – 1.300 Mio.

* roughly estimated

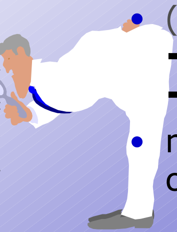
Introduction

- Milk analysers as partially or fully automated systems are widely used in routine milk testing. Why?
- Compared to traditional (chemical and physical) methods, because of
  - ✓ high throughput
  - ✓ high performance (precision characteristics)
  - ✓ data availability and handling
  - ✓ low labour, low costs

Introduction

	Bavaria	Germany	World*
Labs	1	19	800 (2.000)
samples/ year	15 Mio.	40 Mio.	300 – 500 Mio.
analyses	54 Mio.	140 Mio.	1.000 – 1.300 Mio.

ghly estimated



- (interesting) market
  - ➔ different suppliers
  - ➔ different analysers
- need for evaluation to control data quality

- Evaluation work requires high competence and capacities in
  - ✓ Routine method to be validated **AND**
  - ✓ Reference methods and the
  - ✓ Availability of a big range of raw milk samples

➔ this combination is very hard to find

Introduction

- ICAR sets a standard for the approval of milk analysers on an international scale
- ICAR wants to provide
  - ✓ safety
  - ✓ confidence and
  - ✓ cost reduction

  for users as well as for producers of milk analysers AND thus for the whole dairy business (AI and breeding, DHI and single herd management).

General intent of the paper

- to contribute to the understanding, how raw milk analysers are functioning
- to set a standard of minimum requirements
- to avoid work and costs for validation
- to provide expertise and help
- to harmonize approval procedures
- to give safety and make procedures and data transparent

General intent of the paper

„...to define an overall procedure starting from the request for the approval, the procedure for the approval, the description of the technical evaluation needed, providing at the end the elements for a decision on approval.“ (foreword)

General intent of the paper

foreword ➔ scope

- Complies with ISO 8196 (IDF 128)
- Milk of cows, goats, ewes, buffaloes
- Fat, protein, lactose, somatic cell count, urea

Structure of the paper

- Introduction
- Rules of approval
- Course of operations of a technical evaluation
- Report and approval delivery
- Annex A – usual statistical formulas
- Annex B – examples of calculation and presentation

Contents :

2. Rules of the approval

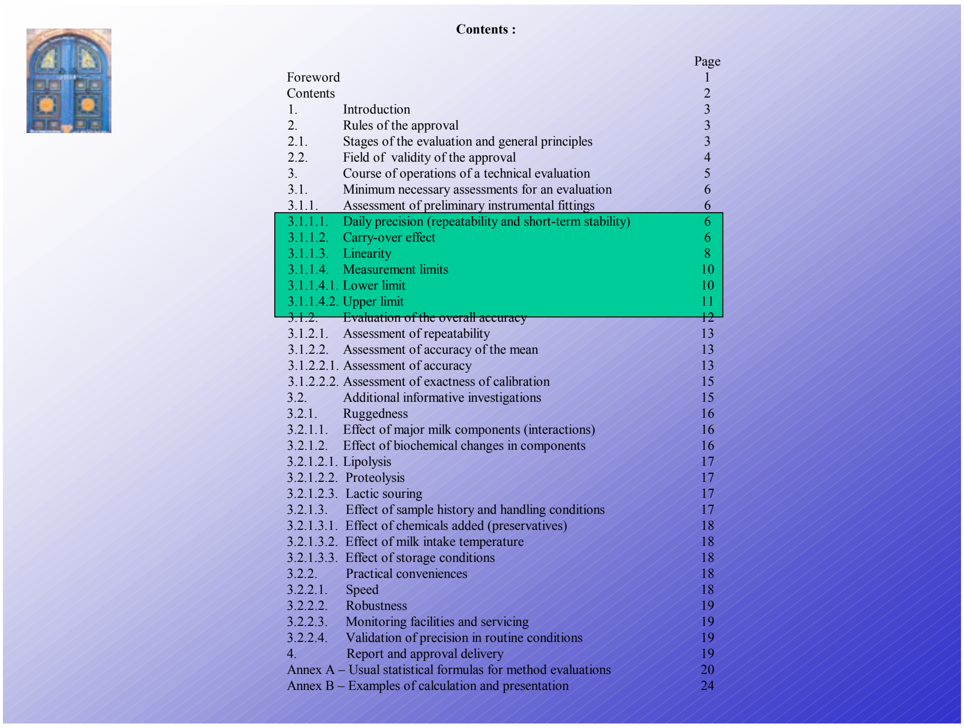
- Phase I – technical assessment by an specialised **expert lab**, accredited or recognised as competent
- Phase II – test under routine conditions in at least **two milk recording labs**
- National approval – Phases I+II on national level, guided by an **official organisation**
- ICAR international approval – decision by **ICAR Board** with technical advice from WG MTL, on the basis of three successful national approvals in ICAR countries

2. Rules of the approval

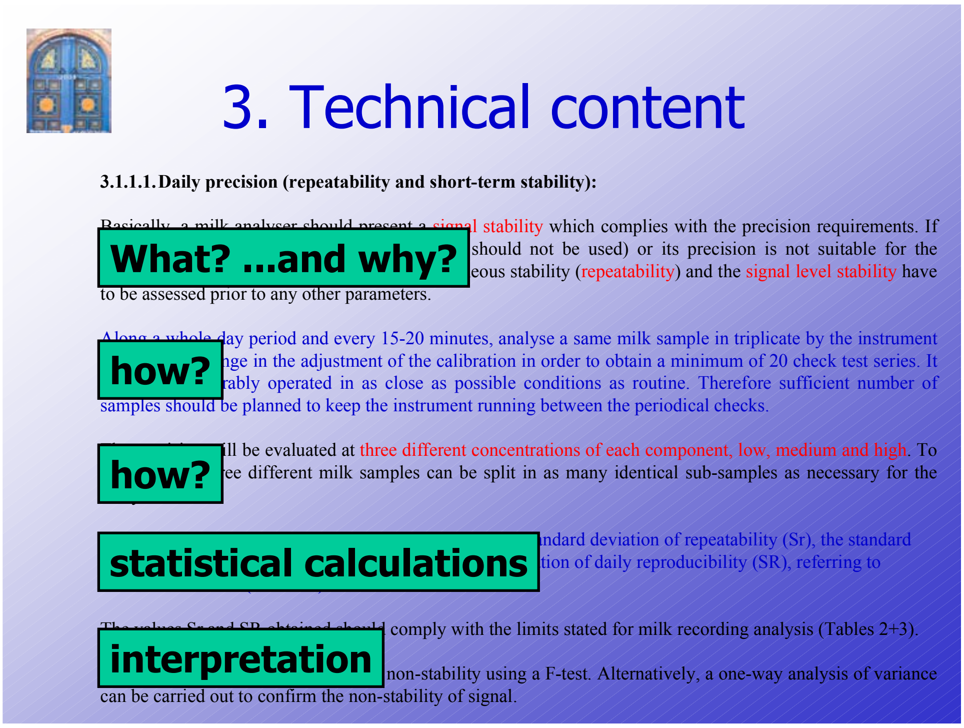
- Validity of approval only for
  - ✓ evaluated application
    - component
    - concentration range
    - animal species
  - ✓ for specific used instrument configuration
    - changed configurations must be checked for influence on precision and accuracy

Minimum ranges to be covered

	cows	goats	ewes	buffaloes	units
Fat	2.0 – 6.0	2.0 – 5.5	5.0 – 10.0	5.0 – 14.0	g/100g
Protein	2.5 – 4.5	2.5 – 5.0	4.0 – 7.0	4.0 – 7.0	g/100g
Lactose	4.0 – 5.5	4.0 – 5.5	4.0 – 5.5	4.0 – 5.5	g/100g
Urea	10.0 – 70.0	10.0 – 70.0	10.0 – 70.0	10.0 – 70.0	g/100g
SCC	0 – 2.000	0 – 2.000	0 – 2.000	0 – 2.000	10^3 cells/ml



Contents :
Page



3. Technical content
3.1.1.1. Daily precision (repeatability and short-term stability):
signal stability which complies with the precision requirements. If
should not be used) or its precision is not suitable for the
eous stability (repeatability) and the signal level stability have
to be assessed prior to any other parameters.
day period and every 15-20 minutes, analyse a same milk sample in triplicate by the instrument
nge in the adjustment of the calibration in order to obtain a minimum of 20 check test series. It
rably operated in as close as possible conditions as routine. Therefore sufficient number of
samples should be planned to keep the instrument running between the periodical checks.
ll be evaluated at three different concentrations of each component, low, medium and high. To
ee different milk samples can be split in as many identical sub-samples as necessary for the
ndard deviation of repeatability (Sr), the standard
tion of daily reproducibility (SR), referring to
comply with the limits stated for milk recording analysis (Tables 2+3).
non-stability using a F-test. Alternatively, a one-way analysis of variance
can be carried out to confirm the non-stability of signal.
What? ...and why?
how?
how?
statistical calculations
interpretation

Contents :

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Contents :

Annex A

„Usual" statistical formulas....

Linearity test :

comparison of a line with a k degree polynomial (reduction of residual error by):

$F_{obs} = ((n-2).Sy,x^2 - (n-k-1).Sy,x^{k\ 2}) / (k-1).Sy,x^{k\ 2} < F_{1-\alpha}$

or $Sy,x / Sy,x^k < ((F_{1-\alpha}.(k-1) + (n-k-1)) / (n-2))^{1/2}$

with: n samples, k polynomial degrees,

k1 = k-1, k2 = q-k-1 and α risk of error.

Annex A

„Usual" statistical formulas....

Linearity test :

Sample or level effect interpreted as linearity compared to repeatability:

$F_{obs} = (n.Ss^2 + Sr^2)/ Sr^2 = (n.(S_{\bar{d}}^{\ 2} - Sr^2/n) + Sr^2)/ Sr^2 = n.S_{\bar{d}}^{\ 2}/ Sr^2 < F_{1-\alpha}$

or $S_{\bar{d}} / Sr < (F_{1-\alpha}/n)^{1/2}$

with: n replicates, $\bar{d}$ = means difference of replicates,
k1 = q-2, k2 = q.(n-1) and α risk of error.

Note: k1 = q-1 when testing the effect of a source of variation with no regression (1 way-ANOVA)

Annex B

Examples of calculation and presentation

ANNEX - EXAMPLES

EVALUATION OF MILK ANALYSERS FOR ICAR APPROVAL

1. Assessment of preliminary instrumental fittings :

1.1. Daily precision : Example of fat analysed by infra red spectroscopy (cf. IDF 141)

Test No q	Replicates	Sum	Mean m	Mean bias d	Test number n	Sum of squares SOS	Variance Var	Within check Sr(i)
1	4,00 4,03 4,01	12,04	4,013	0,008	3	0,000467	0,000233	0,015
2	4,02 4,03 4,02	12,07	4,023	0,018	3	0,000067	0,000033	0,006
3	4,01 4,00 4,00	12,01	4,003	-0,002	3	0,000067	0,000033	0,006
4	3,99 4,00 4,02	12,01	4,003	-0,002	3	0,000467	0,000233	0,015
5	3,99 4,01 4,01	12,01	4,003	-0,002	3	0,000267	0,000133	0,012
6	3,97 3,99 4,00	11,96	3,987	-0,018	3	0,000467	0,000233	0,015
7	4,01 4,00 3,98	11,99	3,997	-0,008	3	0,000467	0,000233	0,015
8	4,02 4,02 3,99	12,03	4,010	0,005	3	0,000600	0,000300	0,017
	4,01							

1	4,00 4,03 4,01	12,04	4,013	0,008	3	0,000467	0,000233	0,015
2	4,02 4,03 4,02	12,07	4,023	0,018	3	0,000067	0,000033	0,006
3	4,01 4,00 4,00	12,01	4,003	-0,002	3	0,000067	0,000033	0,006
4	3,99 4,00 4,02	12,01	4,003	-0,002	3	0,000467	0,000233	0,015
5	3,99 4,01 4,01	12,01	4,003	-0,002	3	0,000267	0,000133	0,012
6	3,97 3,99 4,00	11,96	3,987	-0,018	3	0,000467	0,000233	0,015
7	4,01 4,00 3,98	11,99	3,997	-0,008	3	0,000467	0,000233	0,015
8	4,02 4,02 3,99	12,03	4,010	0,005	3	0,000600	0,000300	0,017
9	4,01 4,00 4,03	12,04	4,013	0,008	3	0,000467	0,000233	0,015
10	3,99 3,99 4,01	11,99	3,997	-0,008	3	0,000267	0,000133	0,012
Sum **Average** **SD**	120,150 4,005	120,150	40,050 4,005 0,010	0,000 0,000 0,010	30	**0,00360** 0,000180	0,00180 0,000180	**0,013**

Check homogeneity of variances within checks :
Thanks to : Cochran Index = Var(max) / Sum of Var < Cochran limit => SD limit = (Cochran limit x Sum of Var)1/2

=> Cochran limit (P=0,95 ; 2 ; 10) = 0,445 **=> SD limit =** 0,0283 never smaller than SD values observed => variance homogeneity admitted

Daily reproducibility :	SR=(Sm2 - Sr2.(1-1/n))1/2	**SR =**	0,015	< 0,028 => conform to IDF 141
Variation between checks :	Sc = (Sm2 - Sr2/n)1/2	**Sc =**	0,007	
Repeatability :	Sr = (Sum Sr(i)2 / q)1/2	**Sr =**	0,013	< 0,014 => conform to IDF 141

Source of variation	**df**	**Sum of squares**	**Mean squares**	**SD**	**F**
Betwen tests	9	0,002950	0,00032778	0,018	1,821
Within tests	20	0,003600	0,00018	0,013	
Total	29	0,00655	0,00022586	0,015	

Conclusions :
1- From Fobs = 1,82 smaller than F0,95 = 2,39, stability is assessed positively : no significant shift of instrument response observed
2- From residual SD =0,013 smaller than Sr=0,014, instrument functioning is assessed positively : no abnormal individual fluctuation

Procedure of application

- Before approval request to ICAR
  - ✓ three national evaluations according to the protocol
  - ✓ reports with compliant results
- ICAR approval procedure (preliminary)
  - ✓ approval request to ICAR Secretary General
  - ✓ registration ➔ submission to WG MTL
  - ✓ examination by expert members – group meeting or standard templates
  - ✓ if negative ➔ full explanation of reasons

Procedure of application

- ICAR approval procedure (preliminary)
  - ✓chairman sums up the positions of group members
  - ✓if positive, report within 2 weeks to the ICAR Secretary General
    - endorsement by ICAR Board
    - addition to the approved instruments´ list
    - publication in ICAR Newsletter and ICAR website
  - ✓if negative, report with helpful remarks for further improvement
  - ✓new approval request may be launched...

Other species than cow

- In general applicable to milk of other species, but some limitations in practise
  - ✓number of expert laboratories
  - ✓number of experienced routine laboratories
  - ✓reference methods (precision data?)
  - ✓cost – benefit – ratio
- ➔ the same arguments apply for why to follow the protocol and to do a „central international ICAR approval procedure"

Acknowledgements

MTL Working Group

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ADR, Germany

Milchprüfring Bayern e.V.

Review of ICAR international proficiency studies from 1996 to 2003

Olivier Leray

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Introduction

Since the implementation of ICAR Reference Laboratory Network in 1996, international proficiency testing schemes are run for raw cow milk at a regular two-yearly frequency for the benefit of laboratory members of the network.

As ICAR trials in cow milk concern fat, protein, lactose, somatic cell count and urea, that review deals with measurements of milk components that are used in milk recording for genetic improvement : fat, protein and somatic cell counting.

Interlaboratory trials may have different objectives since results can be considered from various viewpoints or interest : laboratory (external analytical quality control) and users of data (harmonising organisation in a quality assurance system such as ICAR). Both interests can be met in the framework of ICAR PT schemes and this review lists data for both interests.

Proficiency study protocol

- The organiser to dispatch 10 different milk samples covering the concentration ranges met in routine milk analysers to laboratory participants by international express carriers,
- Laboratories to apply in duplicate reference methods they use to calibrate routine methods (infra red spectrophotometers) for fat and protein and routine methods for somatic cell counting and to return results to the organiser;
- The organiser to apply a standard statistical treatment to results and provide the statistical information about the precision and trueness of every participant and the group of laboratories.

The statistical parameters for the evaluation are the standard deviation of repeatability of the laboratory SL calculated from duplicates, the mean of differences d, the standard deviation of differences Sd and the Euclidian distance D that is equivalent to the overall prediction error with duplicates ($D = d^2+Sd^2$).

Principle of the review

For each trial, each milk component analysed and all the members of ICAR network, values estimated for statistical parameters mentioned above were collected and placed in respective tables and figures. The resulting plots illustrate the scattering of individual precision SL and D and trueness d and Sd.

For fat and protein, the respective positions of the different methods were shown thanks to both illustrations including all the methods used and only the reference methods (without routine methods). For SCC, results of all methods were jointly taken into account since routine fluoro-opto-electronic counters were used in majority. The obtained figures for the statistical parameters revealed clearly the discrepancies where infra red methods were not adequately adapted(milk composition ; F/P internal correlation).

As usual in proficiency testing, abnormal data have occurred which have increased in an abnormal way several parameters. Since it was not possible to reprocess raw data and apply the usual outlier test, abnormal scores were discarded in the second treatment devoted to reference methods. The basis of deletion was the application of Cochran test (1% risk of error) to D values of the group of participants with a 20% limit for discarding (according to IDF 135). Where applied,deletion was done for all the scores of the trial concerned so as to allow proper combined estimates. Too high repeatability was not a deletion criteria.

The comparison of precision figures, as calculated with all the methods and as calculated with only reference methods, thereby excluding outliers, provides a view of the discrepancy due to various errors, mishandling or inappropriate methods (e.g. MIR not fitted for purpose).

Individual lab performance assessment was made possible for the whole period (9-16 trials) by combining/merging statistical parameters and establishing overall scores (meta-analysis). Moreover, individual repeatability and reproducibility values could be calculated for the whole period of time for each participant. This does provide appropriate means for the calculation of the uncertainty of routine test results for internal quality assurance purposes.

Participation of network members

Sixteen international PTs have been organised since 1996 for fat and protein and 9 for somatic cell count with a participation from about 14 to 25 labs in fat, 14 to 26 in protein and 9 to 18 labs in somatic cell counting (SCC).

Though reference methods (Rose-Gottlieb for fat, IDF Standard 1; Kjeldahl for protein, IDF 20) were required, a part of the laboratory participants applied at a lower rate other methods: mid infra red routine methods for fat and protein and non standardised Gerber methods for fat.

A few participants used different methods, reference and routine alternatively, whereas others participated irregularly or for only one of the two yearly rounds per year. In the meantime from 1996, a few countries nominated other laboratory members, new ones or in replacement of the former.

All these elements make the basis for a sound evaluation of overall precision figures unbalanced and give unequal confidence intervals to Sr and SR estimates. As well, individual assessments cumulated over the whole period 1996-2003 (meta-analysis) cannot be considered equivalent with regard to individual lab participation rates, what must be kept in mind when comparing laboratories performances.

Overall precision

In general, precision figures Sr and SR appear significantly larger than those of international standards IDF/ISO. This can be explained by different conditions in trials (laboratory and sample number, concentration ranges, etc) on the one hand and by a different outlier discarding method that points out only

	F	P	SCC
Total scores	249	288	138
Outliers	46	49	27
Outliers %	18%	17%	20%

abnormally high D scores on the other hand. The 20% limit for outlier discarding was often reached for each component fat, protein and somatic cells and the process stopped. Thus respective rates of deletion were 18 %, 17 % and 20 %. It is likely that a few abnormal deviations still remain and hamper precision estimates. Nevertheless, it gives a good indication about the present state of the art.

The high proportion of abnormal scores may constitute a warning to laboratories to be cautious on basic mistakes (i.e. printing/writting, reporting, identification/order, calculation). These mistakes are generally at the origin of strong biases, beside pure analytical errors. It is essential that GLP and AQA recommendations are applied.

Individual scores and overall individual assessment

Each laboratory is expected to monitor the reported scores for every parameter, using the provided tables and control charts and react in due time for its own interest. Averaging over a defined period of time or number of trials (rolling assessment) provides robust estimates of the own lab precision that reflects closer its capabilities.

Lab precision and uncertainty

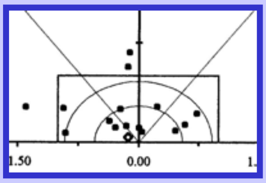
Hence, precision figures calculated SrL and SRL can be used advantageously to determine the uncertainty of routine test results : Uncertainty = $u_{0.975} . [SRL^2 + SrL^2 . (1/n -1)]^{1/2}$. The example of three laboratories with best overall performances for fat shows significant different uncertainty figures. However, uncertainties derived from international method standards are not in all cases met.

Qualification of reference laboratories

Reference materials (RMs) are effective tools for the harmonisation of methods and test results. Defining reference values for such materials is a major operation that necessitates good analytical performance. Through individual lab assessment in international PTs, it is possible to identify and qualify laboratories for that purpose. Data collected since 1996 allow to do so. This is a topical issue for an international quality assurance system, especially in specific areas where reference methods are difficult to perform in every routine laboratory.

Conclusion

International proficiency studies are powerful tools to national and international organisations for the monitoring of routine laboratories and testing laboratories, to identify failures and to improve their performance. It is therefore of major interest for laboratories to participate regularly in proficiency schemes. In the frame of an international reference laboratory network, means are provided to be aware of the state of the art and actual precision of a method and, through scrutinising data, to detect weak points and strive to improve methods via international standardisation.
Secondary use can be developed from collected data that can bring new operational solutions -collective or individual- in the field of the analytical quality assurance for the benefit of both labs and data users. International proficiency schemes can be used by national reference (master) laboratories on a regular rate worldwide, whereas routine testing laboratories can benefit through their respective national laboratories, providinggood linkage and analytical traceability to an international reference system.



International proficiency studies in ICAR Reference Laboratory Network

Review from 1996 to 2003

Olivier Leray, CECALAIT, France

Meeting of ICAR Reference Laboratory Network, 31 May 2004 - ICAR Session Sousse 2004 1

SUMMARY

- General
- Protocol of proficiency studies
- Data treatment in the review
- Participation
- Review of performances : Fat, protein and SCC
  - ➪ Overall precision
  - ➪ individual lab evaluation
- Alternative use of PTs results:
  - ➪ Measurement uncertainty
  - ➪ assignment of reference values

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ICAR International Interlaboratory Proficiency Studies

GENERAL

- **From 1996 to 2003 :**
  - 16 trials for Fat, Protein & Lactose
  - 9 trials for Somatic Cell Counting
  - 8 trials for Urea
- **Dealt with in the review :** Fat, Protein & Somatic Cell Counting

Meeting of ICAR Reference Laboratory Network, 31 May 2004 - ICAR Session Sousse 2004 3

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 (1.5-5.0 % F, 2.6-3.9 % P, 0-1700 .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 (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 ($SRL = \sqrt{SrL^2/2 + \bar{d}^2 + Sd^2}$),

- 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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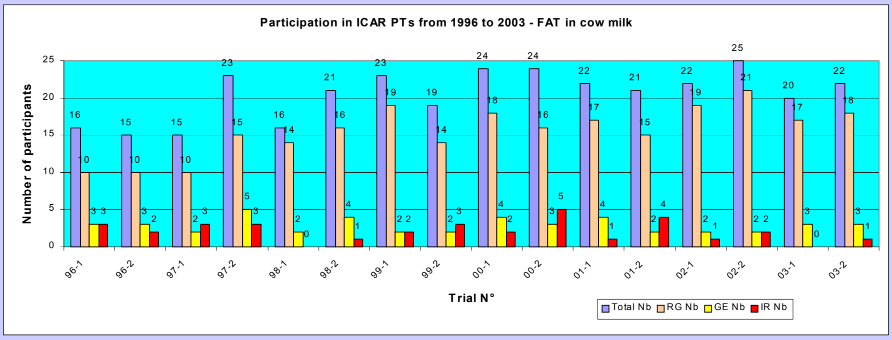
Participation in PTs for FAT

from 1996 to 2003



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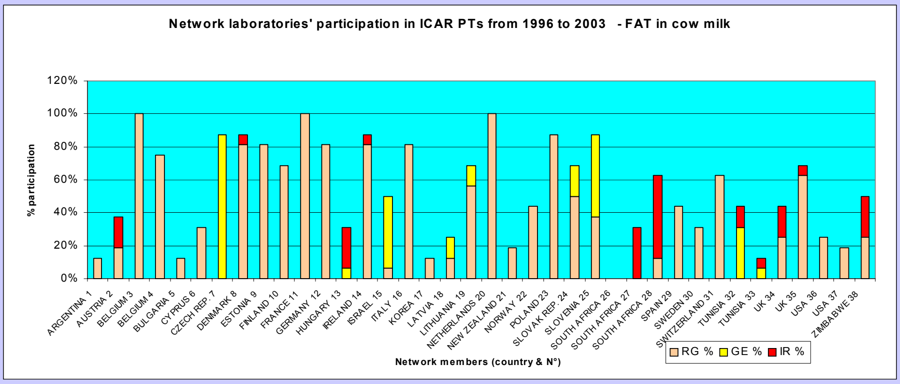
Participation in PTs for FAT from 1996 to 2003



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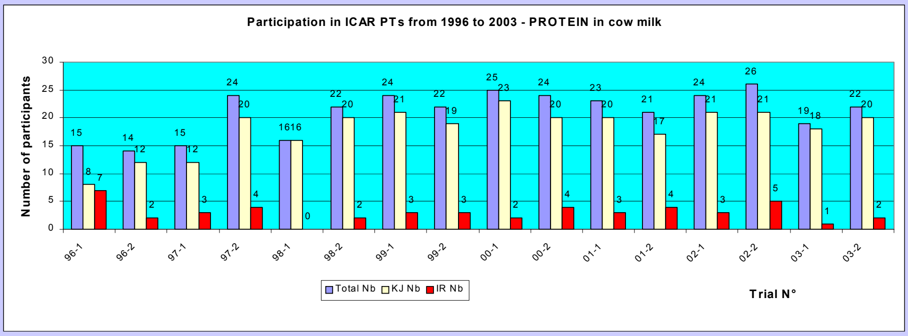
Participation in PTs for PROTEIN from 1996 to 2003



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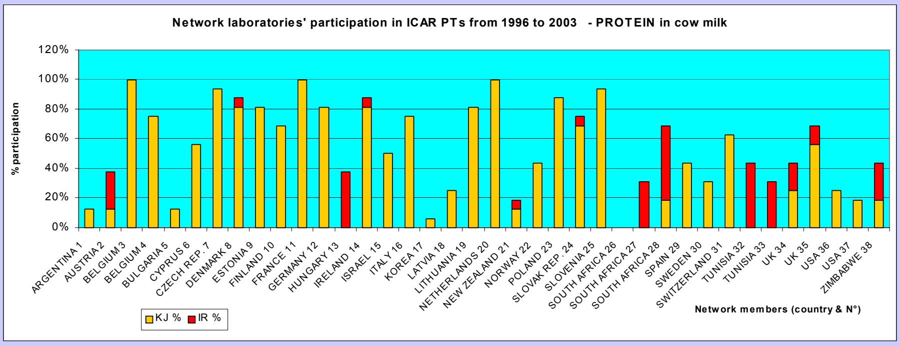
Participation in PTs for PROTEIN from 1996 to 2003



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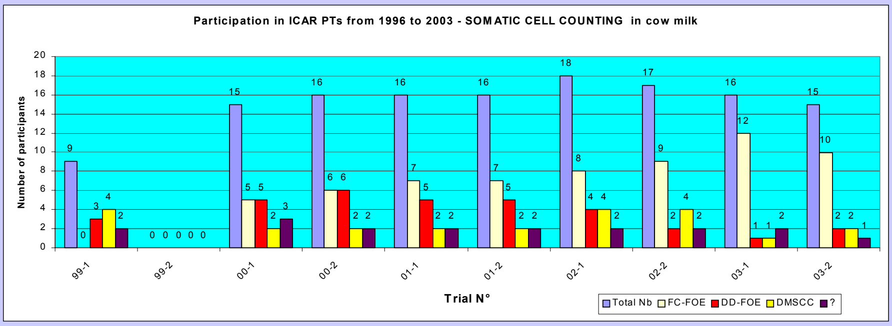
Participation in PTs for SOMATIC CELL COUNTING from 1996 to 2003



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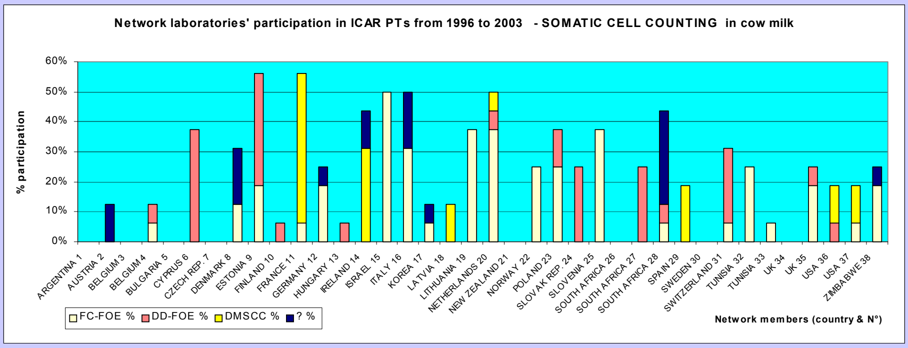
Participation in PTs for SOMATIC CELL COUNTING from 1996 to 2003



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Overview of statistical parameter results with comparing :

- all the methods, all the data

- after discarding routine methods and « outliers »

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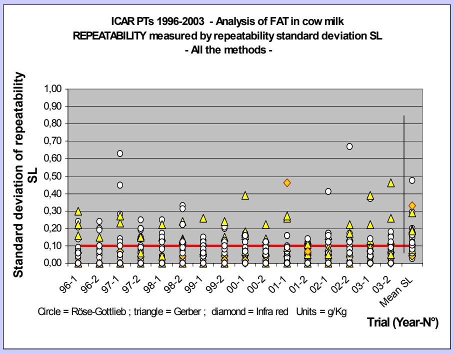
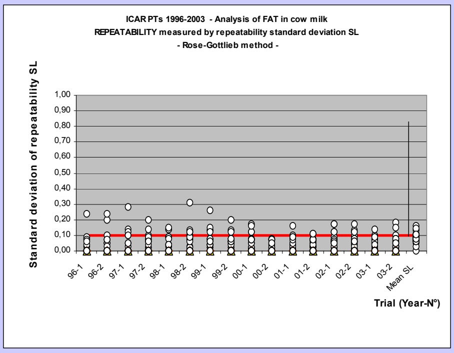
Repeability of FAT measurements





Standard Sr = 0.07 g/Kg

Tolerance limit = 0.10 g/Kg

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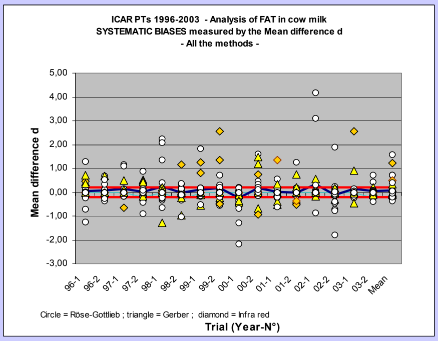
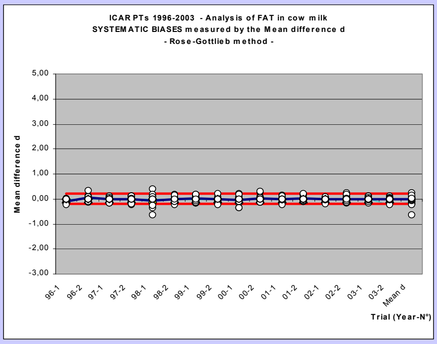
Mean differences $\bar{d}$ for FAT





Standard SR = 0.14 g/Kg

Tolerance limit = +/- 0.20 g/Kg

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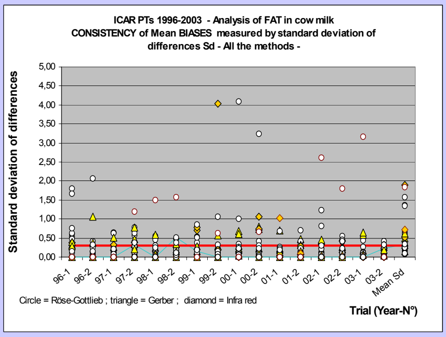
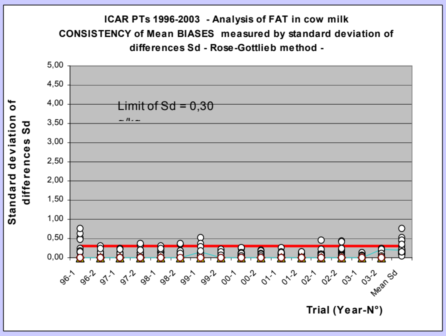
Standard deviation of differences Sd for FAT





Tolerance limit = 0.30 g/Kg

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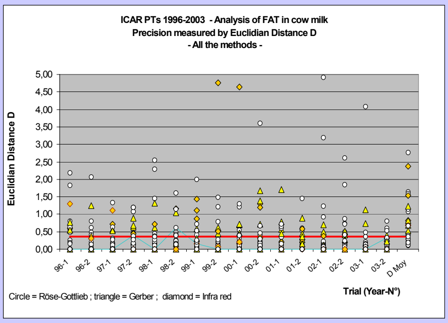
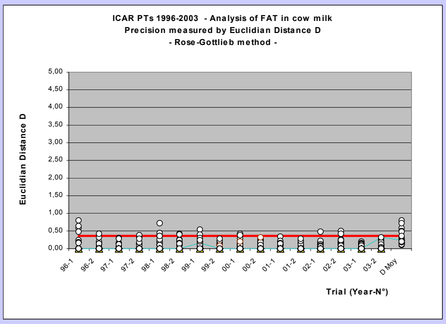
Euclidian distance D (Prediction Error) for FAT





Tolerance limit = 0.36 g/Kg

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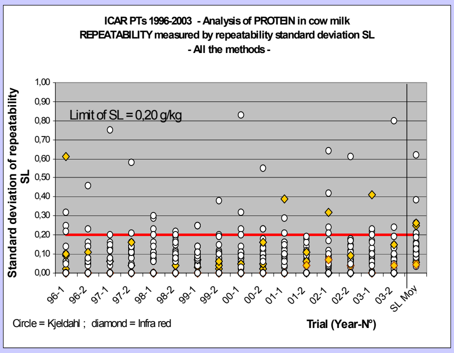
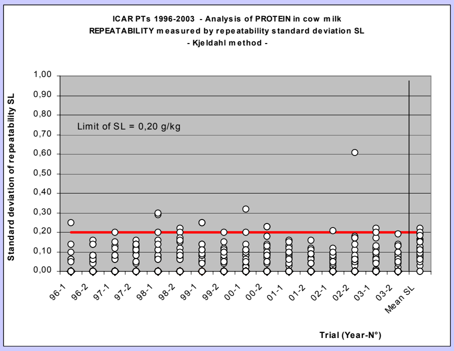
Repeability of PROTEIN measurements





Standard Sr = 0.14 g/Kg

Tolerance limit = 0.18 g/Kg

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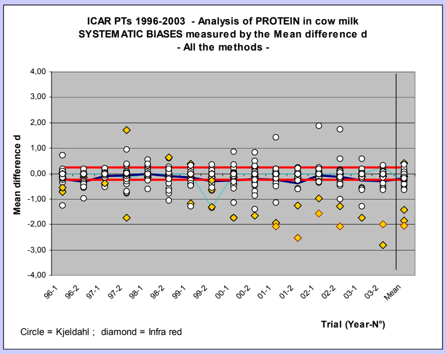
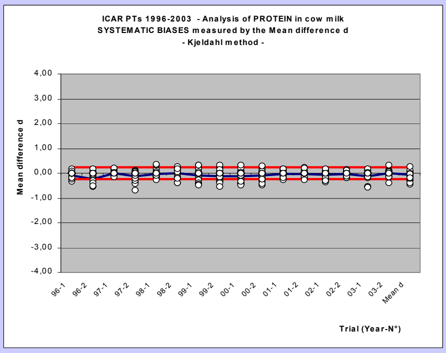
Mean differences $\bar{d}$ for PROTEIN





Standard SR = 0.18 g/Kg

Tolerance limit = +/- 0.25 g/Kg

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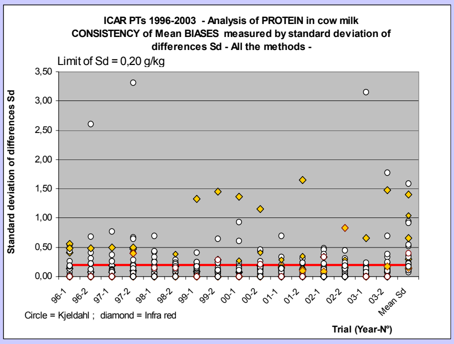
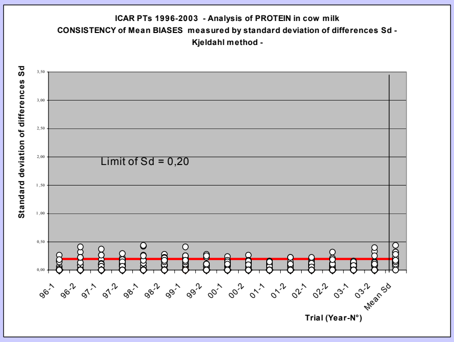
Standard deviation of differences Sd for PROTEIN





Tolerance limit = 0.20 g/Kg

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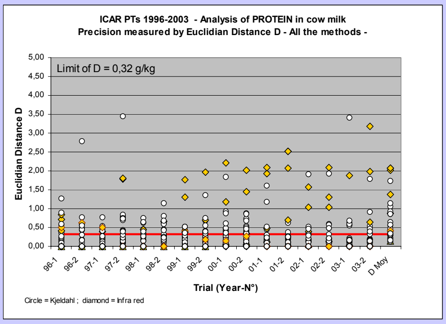
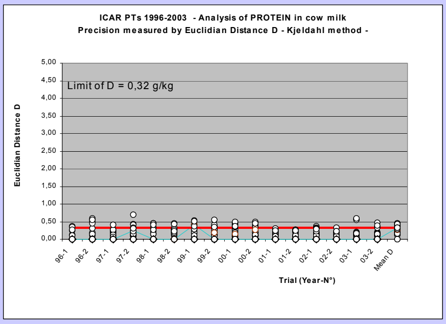
Euclidian distance D (Prediction Error) for PROTEIN





Tolerance limit = 0.32 g/Kg

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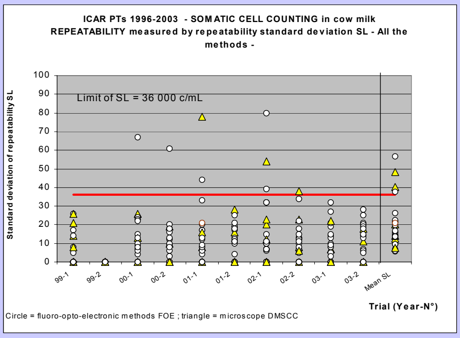
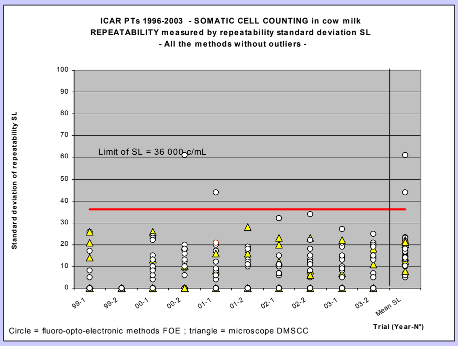
Repeability of SCC measurements





Standard Sr = 20 .1000 cells/mL

Tolerance limit = 36 .1000 cells/mL

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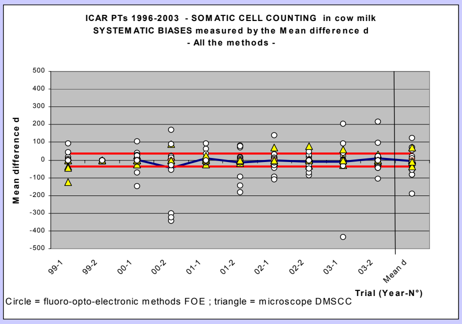
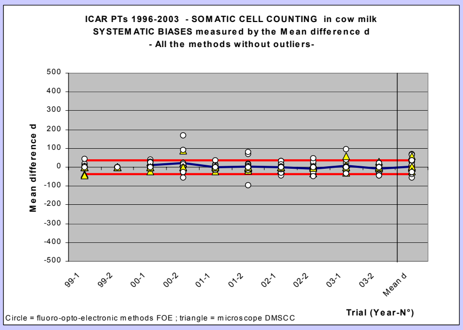
Mean differences $\overline{d}$ for SCC





Standard SR = 50 .1000 cells/mL

Tolerance limit = +/- 35 .1000 cells/mL

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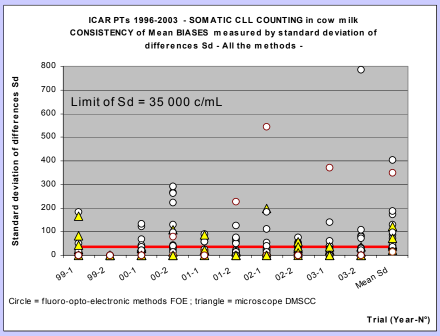
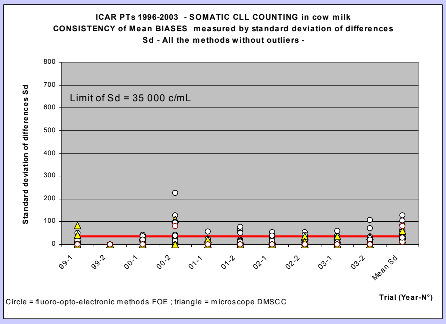
Standard deviation of differences Sd for SCC





Tolerance limit = 35 .1000 cells/mL

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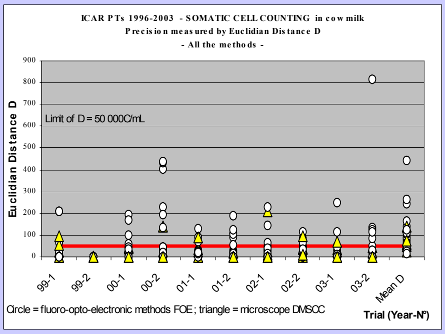
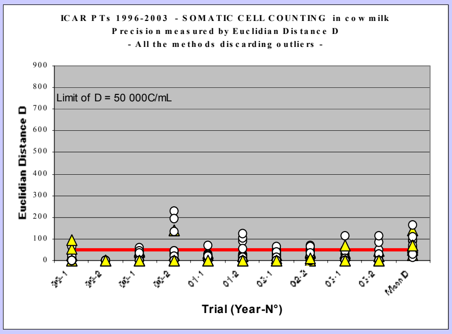
Euclidian distance D (Prediction Error) for SCC





Tolerance limit = 50 .1000 cells/mL

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Precision characteristics of methods within the network

Meta-analysis on 1996-2003

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ICAR PTs 1996-2003 : Overall precision of FAT measurements — IDF 1 : Sr = 0,07 g/Kg and SR = 0,14 g/Kg

Méta-analysis : Discarding outliers on D by Cochran 1% with max. 20%

N° ICAR	96-1	96-2	97-1	97-2	98-1	98-2	99-1	99-2	00-1	00-2	01-1	01-2	02-1	02-2	03-1	03-2	Mean Val
Mean SL	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	0,09
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	-0,01
Mean Sd	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	0,18
Mean D	0,47	0,22	0,18	0,22	0,34	0,22	0,23	0,17	0,20	0,18	0,16	0,15	0,16	0,22	0,12	0,15	0,23
Mean SR	0,47	0,24	0,20	0,23	0,36	0,24	0,24	0,18	0,21	0,19	0,17	0,16	0,18	0,23	0,13	0,16	0,24
Number N	8	8	8	12	11	13	16	11	15	13	14	12	16	17	14	15	203

ICAR PTs 1996-2003 : Overall precision of PROTEIN measurements — IDF 20 : Sr = 0,14 g/Kg and SR = 0,18 g/Kg

Méta-analysis : Discarding outliers on D by Cochran 1% with max. 20%

N° ICAR	96-1	96-2	97-1	97-2	98-1	98-2	99-1	99-2	00-1	00-2	01-1	01-2	02-1	02-2	03-1	03-2	Mean Val
Mean SL	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	0,12
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	-0,07
Mean Sd	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	0,16
Mean D	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	0,27
Mean SR	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	0,29
Number N	6	10	10	16	13	16	21	16	19	16	16	15	17	17	15	16	239

ICAR PTs 1996-2003 : Overall precision of SCC measurements — IDF 148 : Sr = 20 .1000 c/mL and SR = 50 .1000 c/mL

(mean levels approx. 650.000 cells/mL) Méta-analysis : Discarding outliers on D by Cochran 1% with max. 20%

N° ICAR	96-1	96-2	97-1	97-2	98-1	98-2	99-1	99-2	00-1	00-2	01-1	01-2	02-1	02-2	03-1	03-2	Mean Val
Mean SL							18,39		16,77	22,08	17,72	16,35	16,73	16,16	14,73	16,67	17,40
Mean d							-2,00		10,08	20,54	0,23	1,85	-0,21	-7,50	7,85	-6,17	2,74
Mean Sd							43,38		28,61	92,17	24,16	38,17	25,96	29,55	29,10	43,12	44,08
Mean D							51,56		35,31	111,67	29,26	55,94	33,42	40,66	47,79	49,98	55,69
Mean SR							54,37		37,65	114,07	32,19	58,33	35,93	42,89	50,07	51,88	57,88
Number N							7		12	13	13	13	14	14	13	12	111

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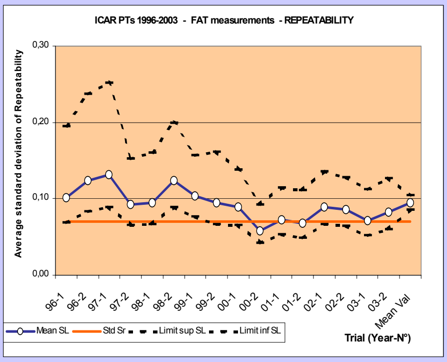
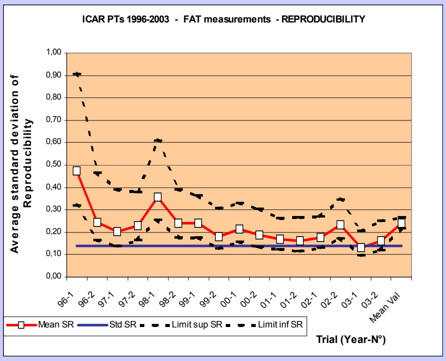
Precision for FAT





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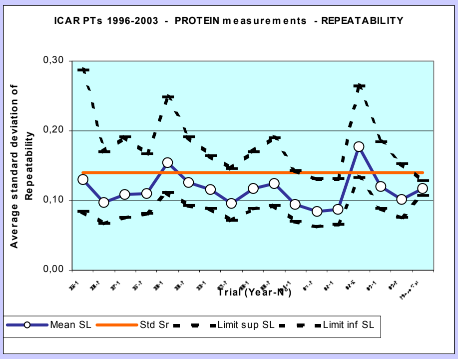
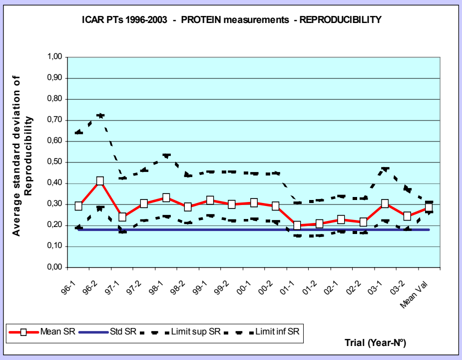
Precision for PROTEIN





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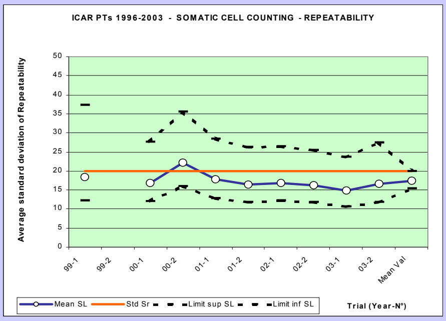
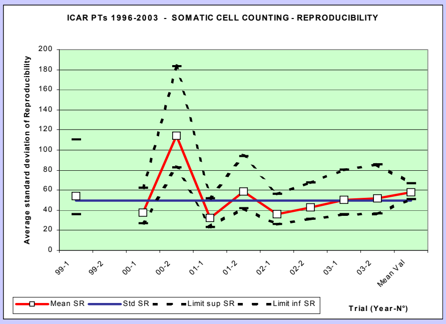
Precision for SOMATIC CELL COUNTING





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Individual laboratory assessment

on a period of time (several trials):

Examples on 1996-2003

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ICAR PTs 1996-2003 : Individual assessment of Precision and Trueness in FAT measurements (Rose-Gottlieb) - Example of Lab 3

N° ICAR	96-1	96-2	97-1	97-2	98-1	98-2	99-1	99-2	00-1	00-2	01-1	01-2	02-1	02-2	03-1	03-2	Mean	Limit
SL (SrL)	0,03	0,20	0,03	0,02	0,04	0,07	0,02	0,06	0,02	0,07	0,02	0,05	0,05	0,05	0,03	0,05	0,07	0,10
Mean d	-0,07	0,09	0,02	0,11	-0,09	0,03	0,03	0,02	0,05	-0,14	-0,06	-0,02	-0,03	-0,15	0,06	-0,02	-0,01	0,20
Sd	0,24	0,13	0,05	0,04	0,15	0,12	0,08	0,13	0,09	0,19	0,07	0,06	0,07	0,07	0,11	0,05	0,12	0,30
D	0,25	0,16	0,05	0,12	0,17	0,12	0,09	0,13	0,10	0,24	0,09	0,06	0,08	0,17	0,13	0,05	0,14	0,36
SRL	0,25	0,21	0,06	0,12	0,18	0,13	0,09	0,14	0,10	0,24	0,09	0,07	0,08	0,17	0,13	0,06	0,15	0,37

ICAR PTs 1996-2003 : Individual assessment of Precision and Trueness in FAT measurements (Rose-Gottlieb) - Example of Lab 11

N° ICAR	96-1	96-2	97-1	97-2	98-1	98-2	99-1	99-2	00-1	00-2	01-1	01-2	02-1	02-2	03-1	03-2	Mean	Limit
SL (SrL)	0,08	0,05	0,14	0,10	0,09	0,09	0,13	0,06	0,08	0,05	0,08	0,08	0,05	0,08	0,07	0,08	0,09	0,10
Mean d	-0,01	0,12	0,03	-0,07	0,00	0,17	-0,16	0,20	0,08	0,10	0,08	0,18	0,02	0,15	-0,23	0,12	0,05	0,20
Sd	0,47	0,08	0,14	0,12	0,13	0,11	0,12	0,03	0,07	0,04	0,08	0,12	0,07	0,06	0,27	0,09	0,16	0,30
D	0,47	0,14	0,14	0,14	0,13	0,20	0,20	0,20	0,11	0,11	0,11	0,22	0,07	0,16	0,35	0,15	0,21	0,36
SRL	0,47	0,15	0,17	0,16	0,14	0,21	0,22	0,21	0,12	0,11	0,13	0,22	0,08	0,17	0,36	0,16	0,22	0,37

ICAR PTs 1996-2003 : Individual assessment of Precision and Trueness in FAT measurements (Rose-Gottlieb) - Example of Lab 20

N° ICAR	96-1	96-2	97-1	97-2	98-1	98-2	99-1	99-2	00-1	00-2	01-1	01-2	02-1	02-2	03-1	03-2	Mean	Limit
SL (SrL)	0,04	0,03	0,05	0,02	0,03	0,06	0,04	0,20	0,02	0,02	0,05	0,04	0,03	0,04	0,05	0,03	0,06	0,10
Mean d	-0,03	-0,02	0,02	0,01	0,08	0,01	-0,01	-0,08	0,00	0,01	-0,01	-0,08	0,02	-0,04	-0,09	0,02	-0,01	0,20
Sd	0,18	0,04	0,05	0,04	0,06	0,09	0,05	0,19	0,05	0,03	0,09	0,09	0,05	0,08	0,10	0,04	0,09	0,30
D	0,18	0,04	0,05	0,04	0,10	0,09	0,05	0,21	0,05	0,03	0,09	0,12	0,05	0,09	0,13	0,04	0,10	0,36
SRL	0,18	0,05	0,06	0,04	0,10	0,10	0,06	0,25	0,05	0,03	0,10	0,12	0,06	0,09	0,14	0,05	0,11	0,37

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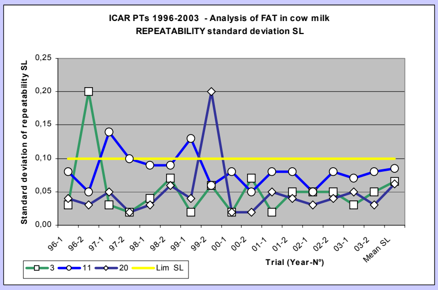

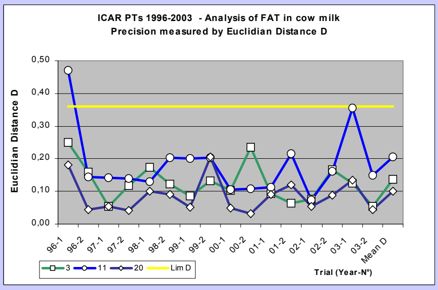

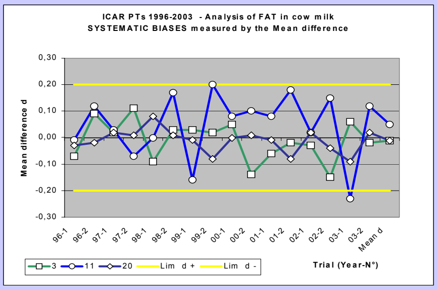

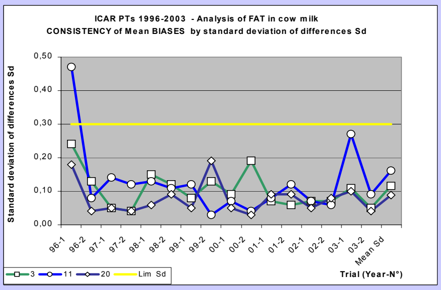

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Determination of uncertainty of measurement results

(Rolling) Square root of r and R mean variances are robust estimates of σrL and σRL of ∞ data:

$$\text{Uncertainty} = \pm u_{0.975} \cdot [SRL^2 + SrL^2 \cdot (1/n - 1)]^{1/2}$$

Example of uncertainty (for FAT results :

N replicate(s)	1	2	3
Lab 3	0,29	0,27	0,27
Lab 11	0,42	0,40	0,40
Lab 20	0,21	0,20	0,19
IS IDF 1	0,27	0,26	0,25
NW 96-03	0,47	0,45	0,44

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

Examples of meta-analysis on 1996-2003

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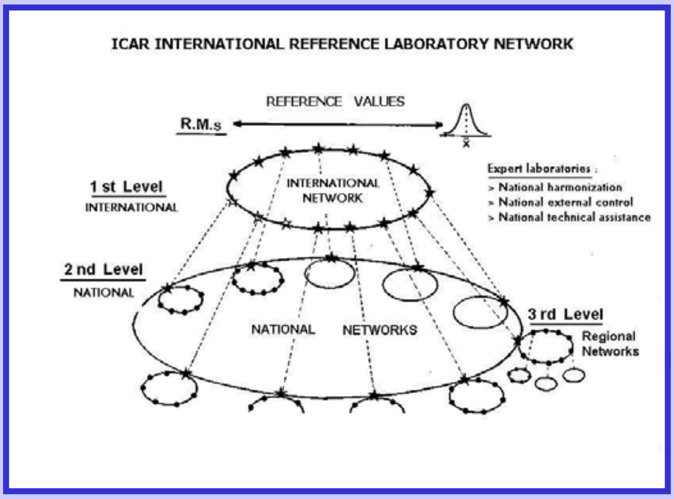

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Average individual performances of network members for FAT between 1996 and 2003 :
(Ranking according to increasing Mean SRL - No outlier discarding - g/Kg)

Rank	Participation	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1	100%	0,06	-0,01	0,04	0,09	0,10	0,11
2	44%	0,06	0,01	0,06	0,11	0,13	0,13
3	100%	0,07	-0,01	0,08	0,12	0,14	0,15
4	81%	0,02	0,09	0,11	0,08	0,16	0,16
5	69%	0,08	-0,02	0,11	0,15	0,17	0,18
6	63%	0,08	-0,05	0,10	0,14	0,18	0,19
7	100%	0,09	0,05	0,12	0,16	0,21	0,22
8	44%	0,10	-0,04	0,17	0,16	0,22	0,23
9	75%	0,05	-0,07	0,21	0,18	0,28	0,28
10	13%	0,11	0,10	0,28	0,18	0,28	0,29
11	88%	0,08	0,01	0,18	0,23	0,29	0,30
12	88%	0,15	-0,08	0,15	0,24	0,29	0,31
13	88%	0,11	-0,06	0,24	0,20	0,31	0,32
14	19%	0,10	0,03	0,14	0,34	0,36	0,37
15	25%	0,08	-0,05	0,16	0,37	0,39	0,40
16	31%	0,09	-0,15	0,23	0,30	0,39	0,40
17	81%	0,11	-0,13	0,20	0,34	0,41	0,42
18	25%	0,17	-0,20	0,12	0,34	0,40	0,42
19	69%	0,17	0,07	0,40	0,25	0,46	0,47
20	94%	0,18	0,16	0,34	0,39	0,53	0,55
21	69%	0,12	-0,20	0,62	0,25	0,67	0,68
22	44%	0,09	0,40	0,58	0,09	0,68	0,68
23	94%	0,19	-0,12	0,62	0,32	0,69	0,70
24	31%	0,20	0,02	0,34	0,66	0,73	0,74
25	50%	0,12	-0,01	0,63	0,49	0,77	0,78
26	81%	0,14	-0,25	0,47	0,59	0,79	0,79
27	31%	0,04	-0,23	0,66	0,51	0,81	0,81
28	50%	0,29	0,32	0,45	0,63	0,82	0,85
29	13%	0,29	0,71	0,90	0,52	1,08	1,10
30	13%	0,48	0,40	1,22	0,64	1,14	1,19
31	13%	0,33	0,51	1,21	0,72	1,23	1,25
32	63%	0,08	-0,35	0,69	1,34	1,53	1,53
33	38%	0,05	0,08	0,77	1,37	1,54	1,54
34	69%	0,17	0,70	1,35	0,61	1,59	1,59
35	19%	1,54	-0,32	0,37	1,57	1,63	1,96
36	31%	0,05	1,23	0,80	1,90	2,37	2,37
37	50%	0,12	1,58	1,41	1,84	2,76	2,76
38	0%						

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Average individual performances of network members for PROTEIN between 1996 and 2003 :
(Ranking according to increasing Mean SRL - No outlier discarding - g/Kg)

Rank	Participation	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1	100%	0,07	0,07	0,07	0,07	0,12	0,13
2	13%	0,08	-0,05	0,08	0,10	0,12	0,14
3	100%	0,07	-0,01	0,11	0,13	0,17	0,18
4	50%	0,16	0,01	0,06	0,12	0,13	0,18
5	44%	0,11	0,00	0,12	0,12	0,16	0,18
6	100%	0,07	0,06	0,16	0,12	0,20	0,21
7	75%	0,08	0,12	0,16	0,11	0,22	0,23
8	6%	0,09	0,02	0,00	0,23	0,23	0,24
9	81%	0,10	-0,09	0,24	0,16	0,29	0,30
10	44%	0,11	-0,14	0,24	0,15	0,31	0,31
11	88%	0,20	-0,10	0,19	0,20	0,29	0,32
12	81%	0,11	-0,25	0,11	0,14	0,31	0,32
13	63%	0,15	-0,21	0,17	0,16	0,30	0,32
14	81%	0,07	-0,07	0,31	0,16	0,35	0,35
15	19%	0,15	-0,19	0,27	0,17	0,34	0,35
16	25%	0,10	-0,12	0,32	0,17	0,35	0,35
17	94%	0,16	-0,21	0,21	0,23	0,37	0,39
18	88%	0,12	-0,29	0,27	0,12	0,39	0,40
19	88%	0,12	0,01	0,37	0,21	0,41	0,42
20	44%	0,10	-0,22	0,13	0,33	0,41	0,42
21	69%	0,26	-0,24	0,22	0,23	0,39	0,43
22	94%	0,13	0,06	0,40	0,18	0,43	0,44
23	31%	0,10	-0,42	0,17	0,11	0,46	0,46
24	38%	0,03	-0,45	0,32	0,14	0,55	0,55
25	69%	0,14	-0,22	0,49	0,22	0,56	0,57
26	13%	0,21	-0,26	0,74	0,52	0,64	0,65
27	44%	0,13	0,36	0,73	0,40	0,87	0,87
28	56%	0,21	-0,47	0,66	0,36	0,86	0,88
29	38%	0,25	0,40	0,78	0,30	0,87	0,89
30	75%	0,39	0,22	0,75	0,55	0,93	0,97
31	75%	0,21	-0,25	0,40	0,94	1,05	1,06
32	25%	0,19	-0,32	0,70	0,92	1,15	1,15
33	69%	0,08	-0,63	0,68	1,04	1,38	1,38
34	19%	0,62	-0,60	0,46	1,59	1,74	1,79
35	31%	0,05	-1,43	0,26	1,40	2,01	2,01
36	44%	0,26	-1,86	0,64	0,66	2,06	2,07
37	31%	0,04	-2,05	0,34	0,14	2,08	2,08
38	0%						

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Average individual performances of network members for SCC between 1996 and 2003 :
(Ranking according to increasing Mean SRL - No outlier discarding - x1000 c/mL)

Rank	Participation	Mean SL	Mean d	S mean d	Mean Sd	Mean D	Mean SRL
1	11%	21,00	-3,00	0,00	13,00	13,34	19,96
2	100%	14,15	2,78	14,19	21,64	25,59	27,48
3	67%	14,54	3,83	18,43	20,21	26,58	28,50
4	56%	21,48	11,20	13,37	18,84	24,97	29,22
5	56%	14,21	-10,20	22,33	17,36	28,36	30,08
6	67%	13,29	-4,00	17,79	23,41	28,77	30,27
7	100%	16,15	3,63	19,68	22,67	29,43	31,57
8	89%	12,91	9,75	22,48	26,82	35,45	36,61
9	22%	14,58	-15,50	20,51	29,55	36,38	37,81
10	22%	12,59	-2,00	46,67	39,20	51,28	52,05
11	44%	26,18	20,75	30,82	37,65	50,60	53,88
12	44%	5,61	-16,75	39,40	38,34	53,99	54,14
13	67%	15,48	-28,83	31,62	40,75	57,66	58,69
14	78%	20,22	-0,29	44,15	46,59	61,98	63,61
15	44%	9,54	-43,50	17,94	47,02	65,91	66,26
16	89%	9,56	-26,25	51,11	53,36	76,31	76,60
17	33%	48,25	-8,00	34,39	70,58	76,38	83,66
18	22%	19,61	56,50	38,89	56,36	84,41	85,54
19	11%	19,00	69,00	0,00	59,00	90,79	91,77
20	89%	21,77	72,13	26,99	70,69	104,10	105,23
21	67%	14,12	-17,17	25,01	103,59	107,46	107,92
22	33%	7,77	-38,00	75,36	98,75	122,40	122,52
23	22%	6,96	-81,00	94,75	87,79	136,95	137,04
24	11%	6,00	-55,00	0,00	129,00	140,24	140,30
25	33%	40,18	69,00	8,54	121,05	139,51	142,38
26	78%	16,85	-55,86	126,24	104,34	166,33	166,76
27	44%	13,69	-80,75	176,97	174,42	245,83	246,02
28	44%	56,76	-10,25	213,30	186,94	263,01	266,06
29	44%	37,72	-188,25	176,13	351,03	426,53	427,36
30	44%	22,34	123,75	147,24	404,07	441,42	441,70
31	0%						
32	0%						
33	0%						
34	0%						
35	0%						
36	0%						
37	0%						
38	0%						

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Conclusions

Observations between 1996 and 2003 have shown :

1- unequal quality in individual performances between labs,

2- presence of abnormal high scores possibly identified as :

* random (poor sample preservation/handling, data reporting),
* systematic (procedure default, calculation, calibration).

➩ Confirmation of needs for particular cautions and GLP to be applied with regard to (PT) samples and method procedure,

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Conclusions

Observations between 1996 and 2003 have shown :

3- Precision characteristics Sr and SR in the network (after deletion of strong outlying scores) higher than those of standards IDF 1, IDF 20 and IDF 148 :

➩ Conditions of Sr and SR determination in PT different from IS,

➩ Wide concentration ranges => effect of high levels,

➩ Question addressed : Appropriateness of IS precision figures

=> updating of IS / PT limits ?

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Conclusions

Collection of data between 1996 and 2003 have demonstrated :

- the ability to define an international anchorage for ICAR countries through international PTs,

and provided the possibility :

- to estimate the closer precision characteristics of methods used which is of interest for collective uses,
- to network members to determine uncertainty related to their test results,
- to identify/constitute reference lab groups to elaborate international consensual reference values for RMs.

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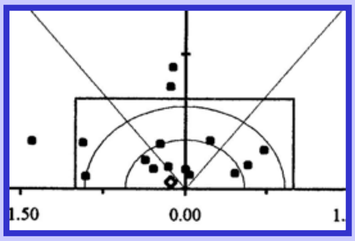

Thank You for your attention !

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Discussion and conclusion

Notes from the discussion : Christian Baumgartner [1] and Olivier Leray [2]

[1] *Milchpruefring Bayern e.V., Germany*
[2] *CECALAIT, France*

During the discussion of the first papers the following points were stressed:

⇨ *Part 1*

- Introduction of the milk recording in sheep, goat and buffalo and current situation in the analytical matter for sheep, goat and buffalo milk analysis :

The three first presentations showed evidence of the need to develop and standardise analytical methods for other species than cow. Especially with regard to analytical methods and practices, it was mentioned that there is a lack of data about reference methods applied in different countries for sheep and goat milk and data collection is not an easy task with regard to the difficulties to get replies to questionnaires from some countries with no centralised organisation or not yet well structured in this area. This lack of data refers to DHI schemes as well as to analytical data and methods. For the time being, analytical data are often obtained with methods validated for cow's milk but not for milks of other species.

The question of how DHI organisations for "minor" species can work in this situation is raised.

Nowadays the response is being brought from international standardisation, which has undertaken to carry out updates of existing international standards thereby accounting for milks of other species. For the time being, the current work deals with small ruminants, i.e. sheep and goat milk.
Moreover, it is stated that is already valid to refer to general standards such as IDF 128. It is reminded that IDF Standard 141 (MIR) already mentions the applicability to milks of other than cow. So do the ICAR guides for DHI laboratories. Until completing the standardisation of reference methods, another response must come from the participation in proficiency schemes for sheep and goat milk to harmonise performances with the (different) methods/procedures presently used. In the future, unique international methods are likely to be adopted by all ICAR countries.

Going into the detail of methods, it is stressed by attending experts that the matrix milk is of important influence on results of reference and routine methods (with regard to different concentration ranges and compositions). This was illustrated in the presentation. Nevertheless, newer routine analysers using the whole mid infra red spectrum (FTMIR) are not as much affected as traditional filter MIR methods. Already the use of filter B for fat (3.5 µm) had drastically reduced the effect of fat composition between the three species (INRA, France, 1982) and it has been demonstrated that the major effect for protein stems from the NPN fraction (approx. 50% urea) and citrates. Those components can now be taken into account in FTMIR calibration.

It is noted that problems with high fat content or other components for reference methods could be dealt with by diluting with water to a "normal" content compared to cow's milk. This way corresponds to one of the two options proposed for standardisation (i.e. half amount of milk for Röse-Gottlieb and Kjeldahl methods).

It is expressed that it would be optimal to calibrate routine analysers with homologous matrices (same type of milk = same species). If this is not possible or feasible, corrections might be applied as obtained from different investigations (e.g. in Germany "ADR-Richtlinie"). In order to achieve appropriate calibrations, it is absolutely necessary that the work undertaken by IDF to adjust the reference methods to the different matrices will be completed.

In general, it is questioned whether WG MTL could monitor and condense all information related to analytical data of other species than cow. To do so, it is suggested ICAR should include experts and countries even outside of ICAR (esp. for buffaloes) and initialise projects and pilots to enhance DHI in these countries. It will be considered as a possible future activity of MTL WG.

It is observed that in many DHI schemes there are thoughts how to reduce costs for analyses. The general feeling is that this must not affect the precision of data and the quality of conclusions. There might be intelligent solutions but everybody must be aware of the consequences.

⇨ *Part 2*

- Protocol for the evaluation of milk analysers for ICAR approval :

The discussion showed that the protocol is appreciated as help. Indeed, approved analysers are an important part in the whole QA process along wiht accreditation of laboratories and regular proficiency testing.

By comparison to the protocol proposed for milk analysers, which is equally applicable to cow, sheep, goat and buffalo milk, the question was raised if there are milk meters and jars with special approval for other species than cow? There is no clear indication from the participants of the meeting. Anyhow, an information on milk meters, jars and other devices can be found on the ICAR website on the area of SC on Recording Devices.

- Review of PTs results from 1996 :

From tables presented, the discussion focused on the question of comparing labs against each other. The speaker explained that for the part concerned the objective was to show how data can be used by a central organisation to constitute groups of labs to participate in assigning consensual values for reference materials. It was not the purpose nor the interest to scrutinise individual performances. The tables showed examples for the methodology to apply where the bases used for selecting laboratories were precision data obtained over the whole period 1996-2003. In practice and as said during the presentation, it should normally be reduced to the most recent significant period. Then a smaller number of trials (4 to 8 trials on 2 years, for instance) using a rolling averaging of individual precision figures should be taken into account. To avoid misuse or misinterpretation, labs and countries will be kept anonymous in any proceedings and other publications.

Nevertheless, if scrutinising of individual performance and following improvements (where it is relevant) are of the responsibility of laboratories in the field with regard to their analytical quality assurance policy, these data can/must be a tool to reference/master laboratories as well as for MTL WG to go into details and to identify possible failures and take further actions for improvement.

The question was asked whether ICAR could provide DHI laboratory accreditation services? The straightforward response could not be given as it depends on the strategy of ICAR in this area. The new existence of ICAR Services may allow to cope with such actions for the administrative aspects and the possibility exists to identify accreditation experts that could operate world wide delegated or simply recognised by ICAR. The question will be if time can be made available for such activities. This is an open door in AQA service activities within the network that might be better linked to action within IDF.

Conclusion of the meeting

The meeting has given rise to satisfaction of every attending person. The diversity of topics dealt with showed a variety of issues and actions undertaken in order to guarantee/improve the security of analytical data for milk recording and the very recent new developments. New ideas and suggestions have sprung out for possible further developments by MTL WG that should be discussed during the forthcoming meeting of MTL WG. It was told it is worthwhile to keep organising such a meeting during Biennial ICAR Sessions in the future. So will be done.

The Chairman thanked the attendance for their active participation, invited every attending person to take part in the meeting of MTL WG to be held from 2.00 p.m. and, for those leaving, addressed his best greetings with a next “Rendez-vous” in 2006.