First ICAR
Reference Laboratory Network Meeting

Interlaken – Switzerland
27 May 2002

MTL WG

ICAR Working Group on
Milk Testing Laboratories

FOREWORD

During the past fifteen years, the world-wide development in animal genetic trade and the calculation of genetic indices based on genetic data from different countries have tremendously increased the need for confidence in genetic values as established in individual ICAR member countries. For ICAR this has justified the development of quality assurance policy, in particular in the field of milk recording analysis.

It was in 1994 at the ICAR Sessions in Ottawa, that it was proposed to initiate the implementation of a Analytical Quality Assurance (AQA) system devoted to milk recording laboratories. The Working Group on Milk Testing Laboratories of ICAR was entrusted to develop such a system, as the activities of this Working Group already focused on various aspects of AQA systems.

Since then, the definition of rules for ICAR countries for an engagement in AQA, guidelines for laboratories and a protocol for the evaluation of milk analysers was produced. This was helped by various surveys on milk recording laboratory practices. Relevant information from this was dispatched to ICAR members. As a further complement, Working Group members do participate in analytical method standardization in IDF/ISO bodies and have recently started evaluating the prerequisites for the application of new analytical tools in milk recording.

The implementation of an international AQA system is based on connecting national reference or pivot laboratories. Each of these individual laboratories is at the same time part of a national analytical network with the routine laboratories in his own country, wherein relevant guidelines and Good Laboratory Practice (GLP) principles are applied. The concept of an international network of reference laboratories was found fully appropriate in light of the aims and has become the corner stone of the AQA system of ICAR.

The concept of an international network of reference laboratories was first experimented by EC at the end of the eighties for EC regulation purposes and is still active today. The general principles for setting up such a network was presented in 1992 by R. Grappin at the Symposium on Quality Assurance in Dairy Laboratories in Sonthofen after a first attempt in 1991 for establishing a similar network within IDF countries. This model was considered suitable for the specific case of milk recording and was decided to be applied from 1996 with the first call for nominations of participants to ICAR National Committees.

At that time, awareness existed that not all ICAR countries were in a position to designate such a “master” laboratory, but it was intended to indicate what was expected in term of missions and activities and invite them to move forward to such kind of an organisation. This could then be concretised later on in the appointment of laboratories which special responsibilities in AQA of routine testing laboratories.

ICAR Reference Laboratory Network has been existing now for six years. Till now, it has mainly been active in the execution of international interlaboratory proficiency studies. Generally communicating by electronic means (e-mail), a need was felt to organise a meeting with representatives of its members. Such a meeting was intended to serve the network objectives, being on the one hand to enhance the internal communication and collaboration within the network and on the other hand to evaluate the efficiency and suitability of AQA systems in place by presentation of examples from some ICAR countries.

This was achieved by a half-day network meeting on 27 May 2002 in Interlaken, Switzerland. Four presentations were made, followed by fruitful talks on network functioning and analytical issues. I invite you to go through the contents of this bundle with the presentations and the report of the discussion during this meeting. From the viewpoint of the participants, the meeting was experienced as very successful, thereby meeting with everybody’s expectation.

Poligny, 8 July 2002

Olivier Leray

Chairman of ICAR Working Group
on Milk Testing Laboratories

CONTENTS

LIST OF PARTICIPANTS

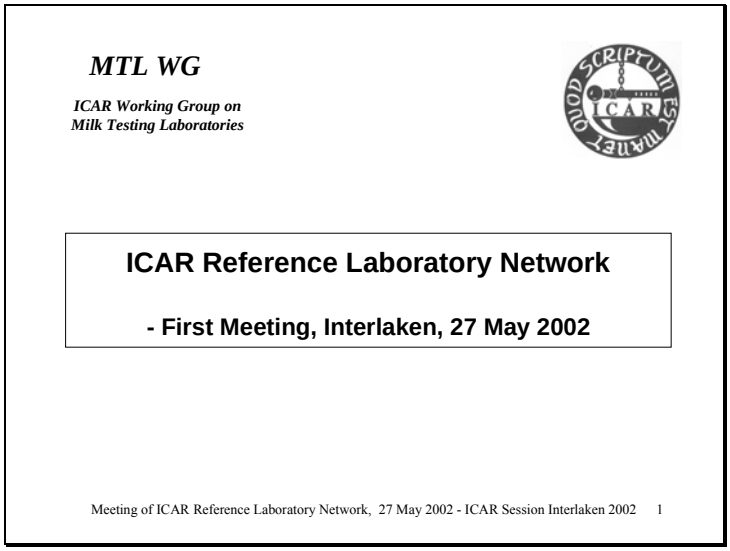
Name	Organisation	City/Town	Country
Oto Hanuš	Research Institute for Cattle Breeding Ltd, Rapotin	Vikyrovice	Czech Republic
Per Sigsgaard	Foss Electric A/S	Hillerød	Denmark
Tove Asmussen	Lattec I/S	Hillerød	Denmark
Toomas Murulo	Animal Recording Centre	Tartu	Estonia
Mart Kuresoo	Animal Recording Centre	Tartu	Estonia
Olivier Leray	Cecalait	Poligny	France
Christian Baumgartner	Milchprüfring Bayern e.V.	München	Germany
Tibor Bajkai	Livestock Performance Testing Ltd.	Gödöllő	Hungary
Ugo Paggi	LSL - AIA	Maccarese	Italy
Laura Monaco	LSL - AIA	Maccarese	Italy
Romas Petruškevičius	Pieno Tyrimai	Kaunus	Lithuania
Harrie van den Bijgaart	Netherlands Milk Control Station	Zutphen	Netherlands
Des Johnston	Livestock Improvement Corporation	Hamilton	New Zealand
Egil Brenne	TINE Norwegian Dairies	Oslo	Norway
Maria Brzozovska	National Animal Breeding Centre	Pruszkow	Poland
Slavica Golc Teger	University of Ljubljana	Domzale	Slovenia
José Tirso Yuste Jordan	Ministerio de Agricultura, Pesca y Alimentación	Madrid	Spain
Thomas Berger	FAM	Bern	Switzerland
Tomas Rydstedt	Steins Laboratorium	Jönköping	Sweden
Hanspeter Roth	SFZV	Zollikofen	Switzerland
John Rhoads	Eastern Laboratory Services	Fairlawn, Ohio	United States
Paul Miller	National DHIA	Columbus, Ohio	United States

Role and Objectives of the Network and Evolution since 1996

Olivier Leray
Cecalait, Poligny (FR)

see enclosed presentation hand-out

Dia 1



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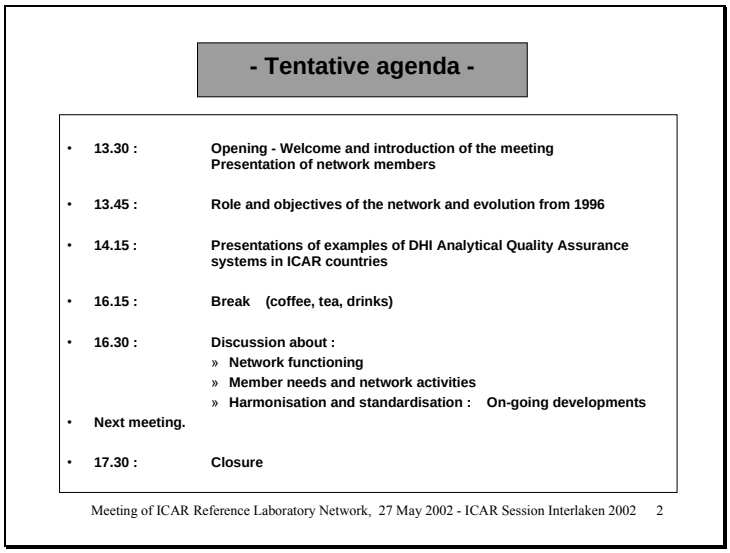

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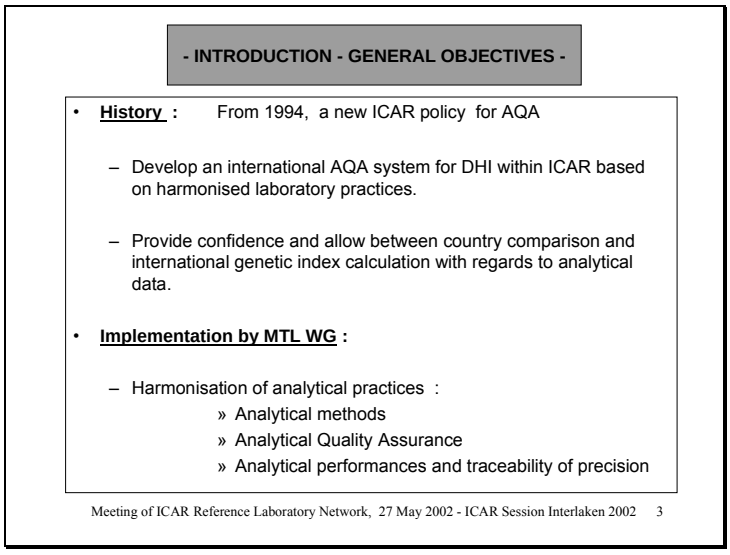

Dia 4

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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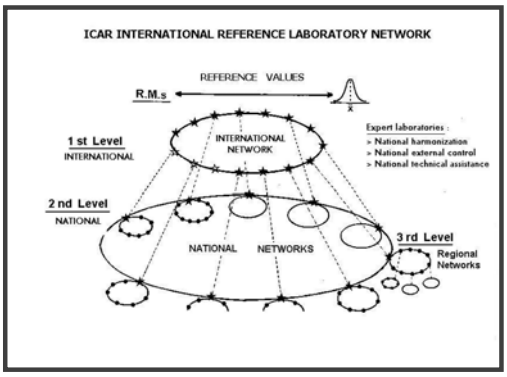
THEORETICAL STRUCTURE



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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
Members and respective missions/activities

ICAR Reference Laboratory Network — Composition and national roles at the date of 27 May 2002

COUNTRY	TOWN	NAME	NRTO	RMS	MLCC	TLT	IAM	EAMI	RAM	NRCA	DHIA	Payment	Other	Member
AUSTRIA	ST MICHAEL	Mischpfriking Süd									c			+
BELGIUM	MELLE	C.L.O. - Gent / O.V.K.	c	c	c	c	c	c	c	c	c	c		+
BELGIUM	GEMBLOUX	CRAG - BGPA	c	c	c	c	c		c	c				+
BULGARIA	SLIVEN	L.A.M.L.G.	c	c	c	c	c							+
CYPRUS	NICOSIA	C.M.I.O.									c g s	c g s		+
CZECH REPUBLIC	VINYROVICE	K.I.C.B.	c	c	c	c	c	c	c					+
DENMARK	HOLSTEBRO	STEINS A/S	c	c	c	c	c		c				c	+
ESTONIA	TARTU	E.A.R.C.	c	c			c			c				+
FINLAND	SAALO	SAALO R&D	c			c	c		c					+
FRANCE	POLIGNY	GECALAIT	c g	c g s	c g s	c g s	c g s	c g s	c g s	c g s				+
GERMANY	GUSTROW	E.L.D.	c								c	c		+
HUNGARY	GODOLLO	L.P.T. Ltd			c		c							+
IRELAND	TERMOY	D.P.S.C. Teagasc	c	c	c	c	c		c					+
ISRAEL	CAESARIA	C.G.A.	c g s	c g s	c g s		c g s	c g s		c				+
ITALY	MACCARESE	AIA-LSL	c g s b	c g s b	c g s b	c g s b	c s b	c g s b		c g s b				+
KOREA	SEOYANG SHI	K.L.C.F.			c	c	c							+
LATVIA	RIGA	V.M.D.C.	c	c		c	c							+
LITHUANIA	KAUNAS	Pieno Tyrimai	c	c	c		c			c g s	c	c		+
NETHERLANDS	SUTPHEN	M.C.S.	c	c		c	c		c		c	c		+
NEW ZEALAND	HAMILTON	N.M.A.C.									c			+
NORWAY	OSLO	T.N.M. BA		c g	c g	c g	c g	c g	c g					+
POLAND	PRUSZKOW	C.S.H.Z.		c			c			c				+
SLOVAK REPUBLIC	NITRA	S.I.A.P.		c		c	c		c					+
SLOVENIA	DOMZALE	Ljubljana University	c	c	c g s	c g s	c g s	c g s	c g s					+
SOUTH AFRICA	ELSENBURG	ANRI. D.L.		c			c		c					+
SOUTH AFRICA	KLAPMSFONTEIN	N.I.D.L.			c						c			+
SOUTH AFRICA	IRENE	TAURUS M.L.			c									+
SPAIN	SANTANDER	Lab. Agrcal. Santand.	c											+
SWEDEN	LUND	S.D.A.-S.D.I.	c	c	c	c	c		c					+
SWITZERLAND	BERN	S.F.R.I.	c g s	c		c	c g s	c g s	c g s					+
TUNISIA	SIDI THABET	L.C.P.									c g s			+
TUNISIA	RIJA	L.C.P.									c g s			+
UNITED KINGDOM	WOLVERHAMPTON	ADAS Lab.		c										+
UNITED KINGDOM	NEWBURY	SN Merit Ltd									c			+
USA	FAIRLAWN	Eastern Lab Services		c	c									+
USA	ITHACA	Dairy One M. A. Lab		c	c				c					+
ZIMBABWE	HARARE	D.S.A.	c			c	c							+
		Number	**20**	**23**	**19**	**19**	**23**	**8**	**15**	**8**	**11**	**5**	**1**	**37**
		Percentage	**54**	**62**	**51**	**51**	**62**	**22**	**41**	**22**	**30**	**14**	**3**	**100**

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

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Dia 8

ICAR Reference Laboratory Network
- Evolution since 1998 -

Evolution of the composition and national roles from 1998 to 2002

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**

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

Evolution of the proportions of national roles from 1998 to 2002

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

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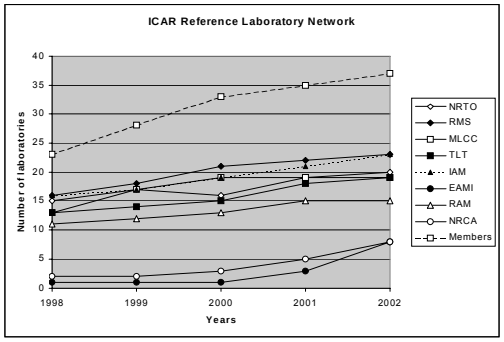
Evolution of membership and missions/activities from 1998 to 2002



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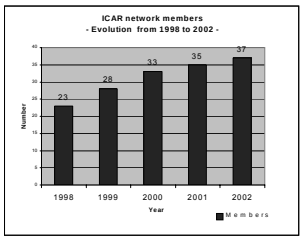
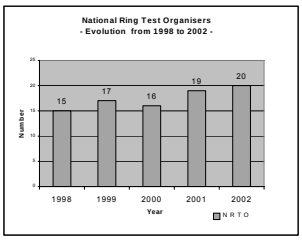
Dia 10

Evolution of membership and missions/activities from 1998 to 2002





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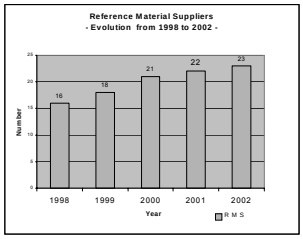
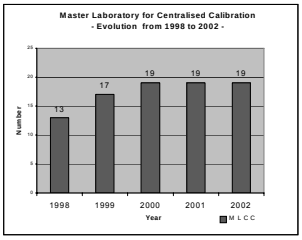
Evolution of membership and missions/activities from 1998 to 2002





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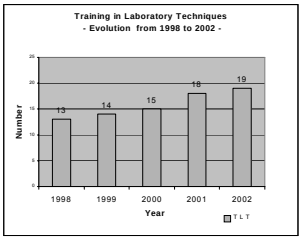
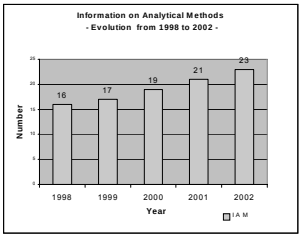
Dia 12

Evolution of membership and missions/activities from 1998 to 2002





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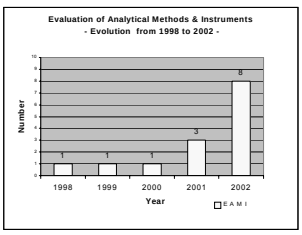
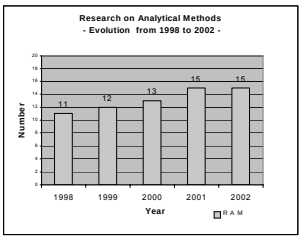
Dia 13

Evolution of membership and missions/activities from 1998 to 2002





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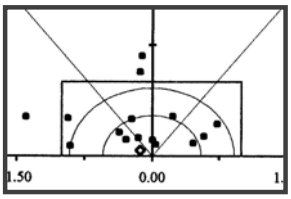
ICAR International Interlaboratory Proficiency Studies



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ICAR International Interlaboratory Proficiency Studies

Cow milk analysis

- **From 1996 to 2001 :**
  - » 12 trials for fat+ protein+ lactose
  - » 5 trials for somatic cell counting
  - » 4 trials for urea
- **Trial organiser :** CECALAIT (Poligny, France)
- **Convention with ICAR :** Definition of experimental conditions and design.

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ICAR International Interlaboratory Proficiency Studies

Sheep and goat milk analysis

- **Launching in Autumn 2002**
  - **Frequency :** Twice per year for each species
  - **Milk compounds :** F, P, L, SCC, Urea
  - **organisation / design :** Same as for cow milk
- **Trial organiser :** LSL-AIA (Maccarese-Rome, Italy)
- **Convention with ICAR :** Definition of experimental conditions and design.

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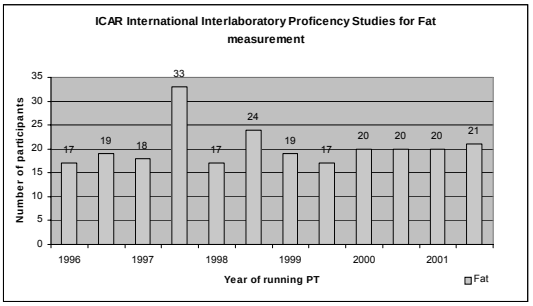
ICAR International Interlaboratory Proficiency Studies



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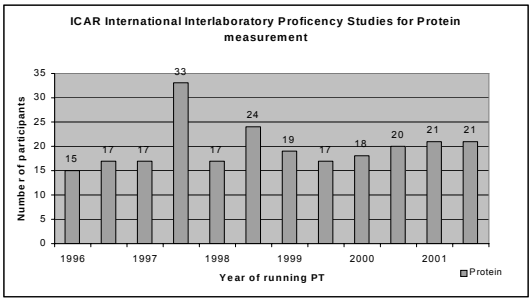
ICAR International Interlaboratory Proficiency Studies



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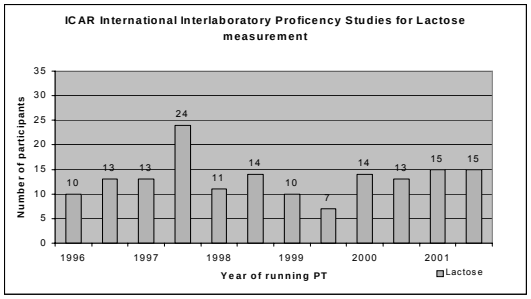
ICAR International Interlaboratory Proficiency Studies



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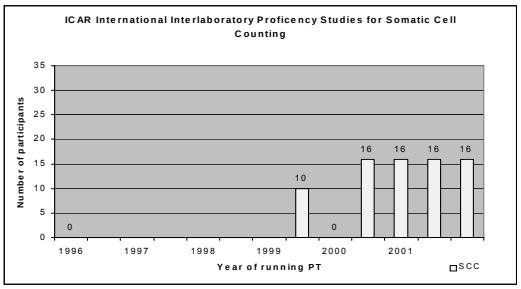
ICAR International Interlaboratory Proficiency Studies



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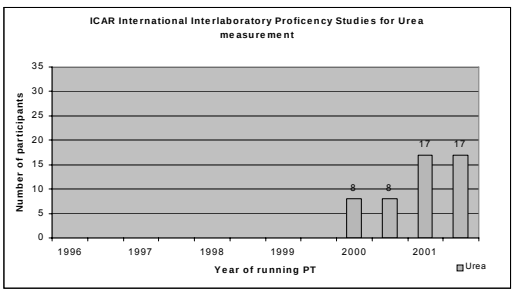
Dia 21

ICAR International Interlaboratory Proficiency Studies



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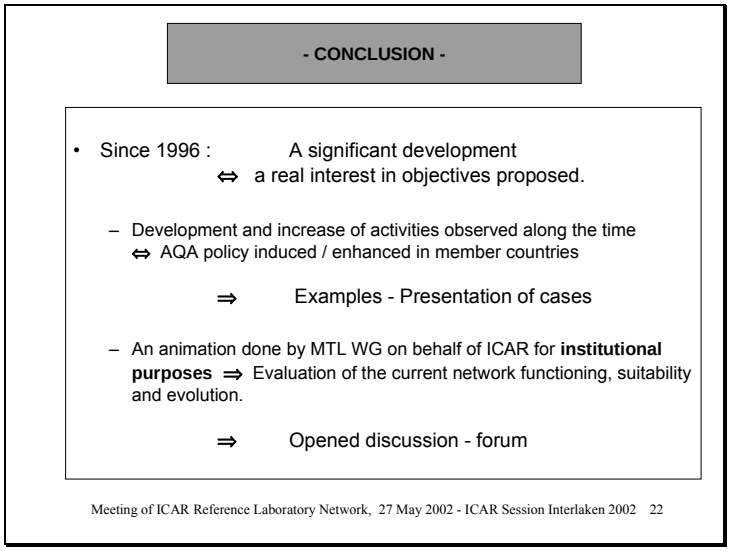

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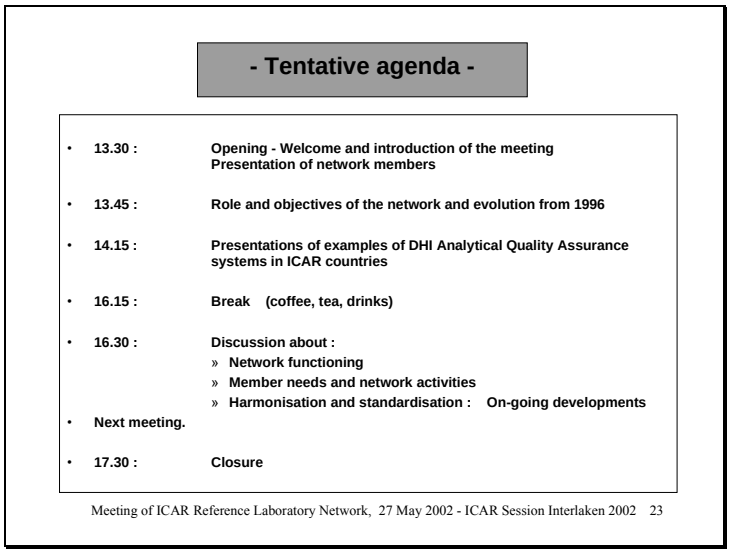

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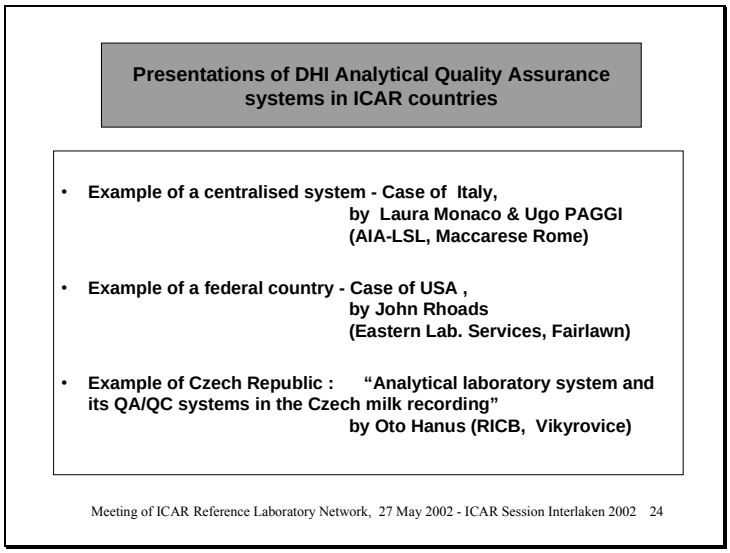

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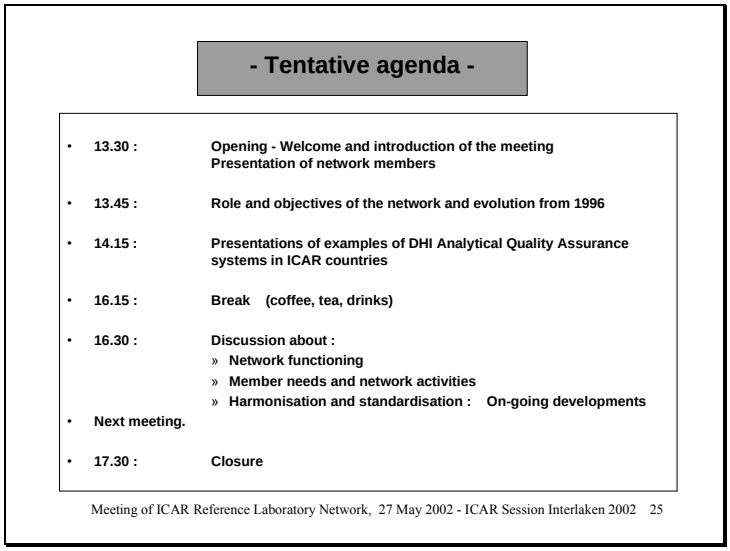
- Tentative agenda -
• 13.30 : Opening - Welcome and introduction of the meeting
Presentation of network members
• 13.45 : Role and objectives of the network and evolution from 1996
• 14.15 : Presentations of examples of DHI Analytical Quality Assurance systems in ICAR countries
• 16.15 : Break (coffee, tea, drinks)
• 16.30 : Discussion about :
» Network functioning
» Member needs and network activities
» Harmonisation and standardisation : On-going developments
• Next meeting.
• 17.30 : Closure
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DHIA Analytical Quality Management System – Example of a Centralised System

Laura Monaco, Silvia Orlandini, Ugo Paggi
A.I.A., Laboratorio Standard Latte, Maccarese (IT)

see enclosed presentation hand-out

Discussion

Miller: Are the same instruments applied for cows and goat milk?

Paggi: Yes, but samples are analysed on separate channels with different calibration settings. For goat and sheep milk specific calibrations are made.

Brenne: In Norway the cows milk calibration is also applied for goat milk, but with a bias correction.

Dia 1

DHIA Analytical Quality Management System

Example of a Centralised System

LABORATORIO STANDARD LATTE

ASSOCIAZIONE ITALIANA ALLEVATORI (A.I.A.)

Laura Monaco – Silvia Orlandini – Ugo Paggi

Dia 2

MILK YIELD YEAR 2000

EUROPEAN UNION 114.604.000

ITALY 10.299.000 (8,99%)

FARMS 74.876

MILK COWS 2.172.000

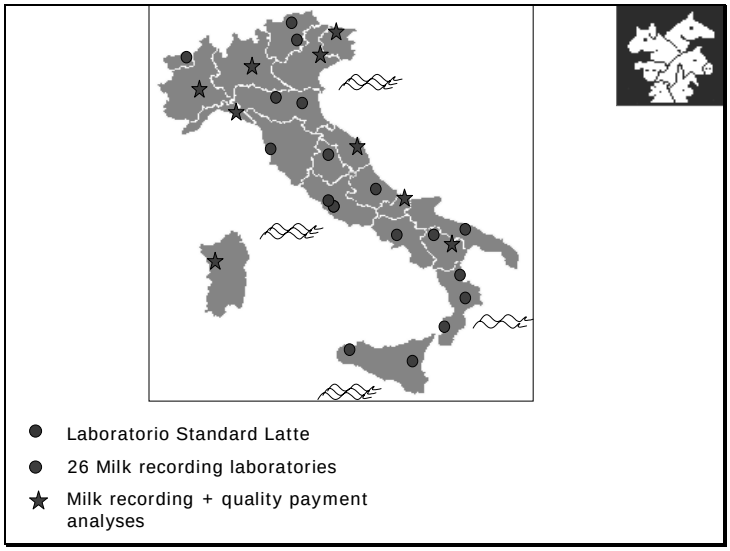
AVERAGE N.OF COWS PER FARM 29

Dia 3

MILK RECORDING (YEAR 2001)

	MILK COWS	YIELD Kg (SD)	FAT g/100g (SD)	PROT. g/100g (SD)
ITALIAN FRESIAN	1.044.670	8.524 (± 2.003)	3,53 (± 0,46)	3,21 (± 0,20)
BROWN	132.868	6.172 (± 1.731)	3,89 (± 0,44)	3,42 (± 0,22)
ITALIAN SIMMENTHAL	45.526	5.847 (± 1.552)	3,89 (± 0,42)	3,39 (± 0,20)
OTHER BREED	67.359	===	===	===
TOTAL	**1.290.423**	**7.925** (± 2.310)	**3,58** (± 0,49)	**3,24** (± 0,22)

Dia 4



Dia 5

YEAR 2001

TOTAL N. ANALYSES 8.700.000

PARAMETERS: FAT, PROTEIN AND SOMATIC CELL COUNT

26 LABS - MAX. 3.500.000 - MIN. 11.000

Dia 6

LABORATORIO STANDARD LATTE

- **Founded in 1990 with contributions from the Italian Ministry of Agriculture.**
- **Coordinates e standardizes the activities, controlling the quality assurance of 26 Milk Laboratories.**
- **The quality assurance is continuously verified and documented to provide improvement.**

www.aia.it/lsl

Dia 7

TOOLS FOR QUALITY ASSURANCE OF ANALYSES

- FIRST TOOL: STANDARD PROCEDURES
- SECOND TOOL: TRAINING AND UPDATING OF PERSONNEL
- THIRD TOOL: AVAILABILITY OF REFERENCE MATERIALS
- FOURTH TOOL: CONTROLS AND AUDITING

Dia 8

FIRST TOOL: PROCEDURES

FAT AND PROTEINS CONTENT (IR)

- STORAGE OF SAMPLES
- EFFICIENCY OF IR INSTRUMENTS
- ANALYTICAL ACCURACY CHECKING

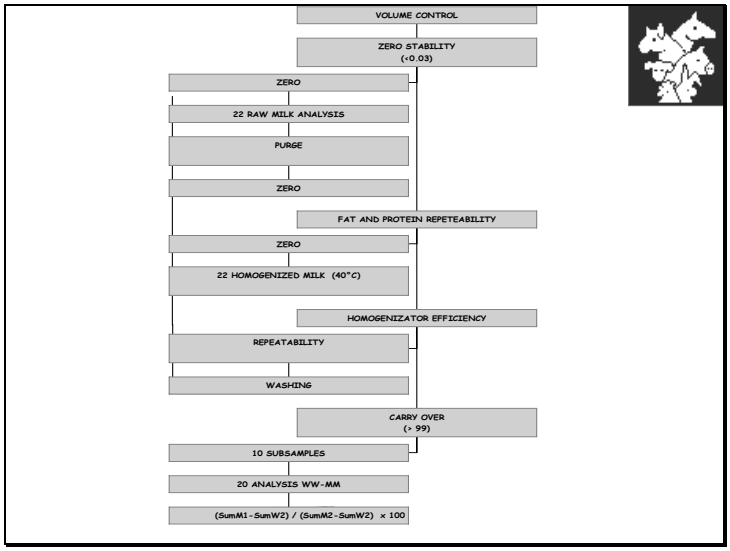
SOMATIC CELL CONTENT

Dia 9

EFFICIENCY OF IR INSTRUMENTS
(FIL/IDF 141 C 2000)

- EFFICIENCY OF HOMOGENIZER
- CARRY OVER
- REPEATABILITY
- ZERO STABILITY
- CHECKING VOLUME SAMPLING

Dia 10



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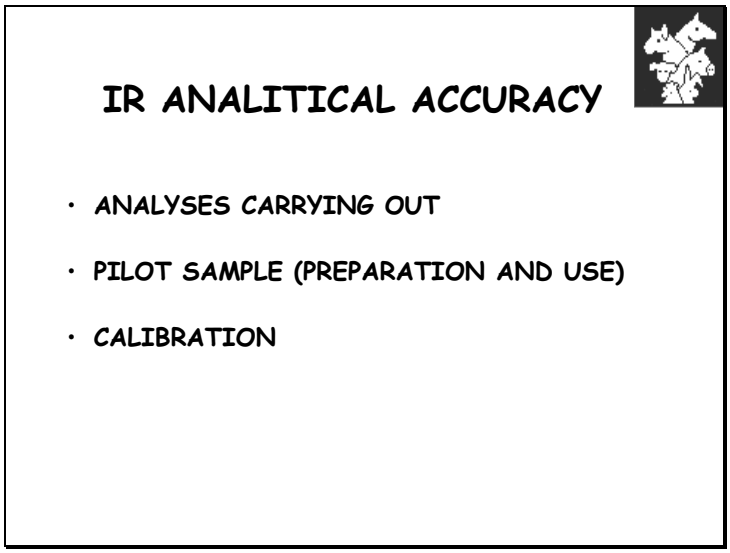
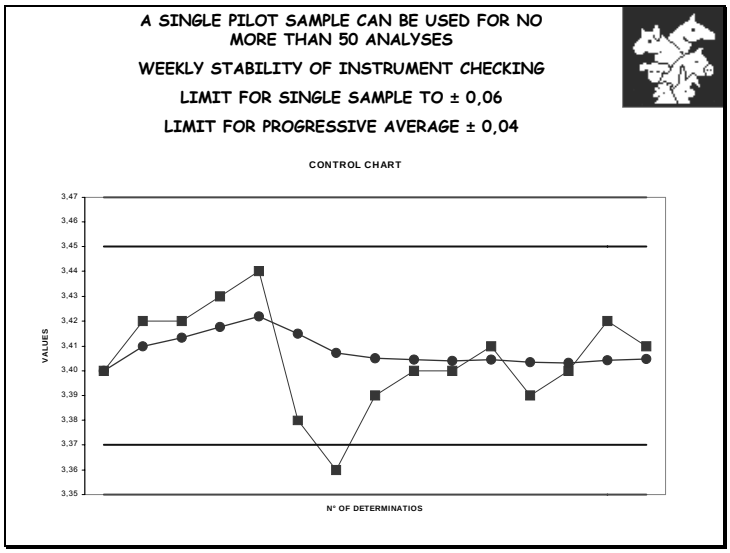


Dia 12



Dia 13

SECOND TOOL: PERSONNEL

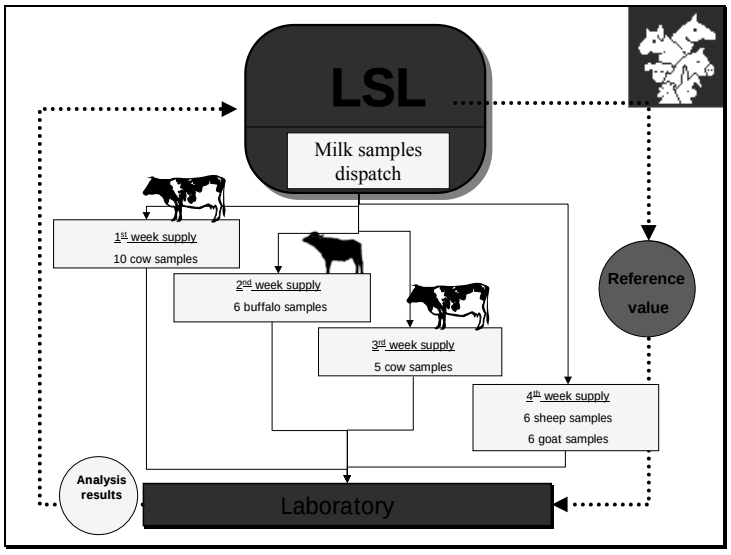
- TRAINING – QUALIFICATION TEST
- MANDATORY YEARLY UPDATING

Dia 14

THIRD TOOL

REFERENCE MATERIAL

Dia 15



Dia 16

THIRD TOOL : REFERENCE MATERIAL (SCC)

EVERY THREE MONTH THE 26 LABS RECEIVE:

- 4 COW MILK SAMPLES - DIFFERENT LEVELS OF SOMATIC CELL CONTENT (720 VIALS). THE LABS MUST CONTROL THE INSTRUMENT CALIBRATION EVERY 15 DAYS;
- COW MILK SAMPLES AS PILOT SAMPLES FOR SOMATIC CELL COUNTS (930 VIALS)

Dia 17

FOURTH TOOLS :

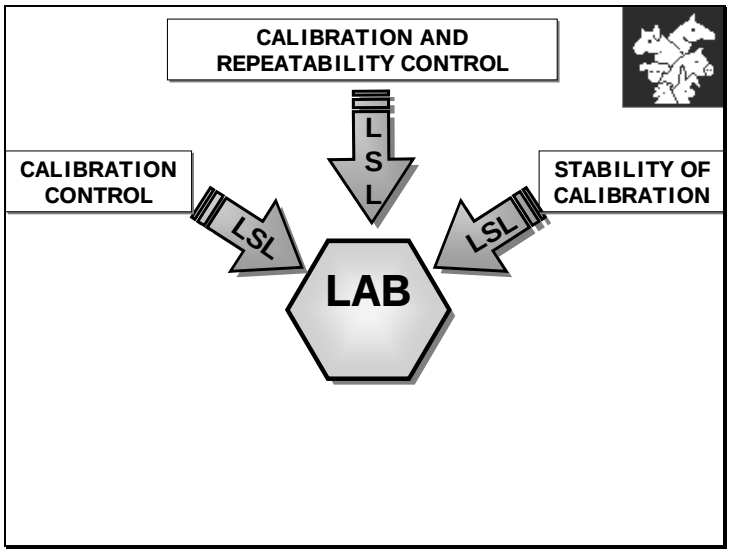
AUDITING

- AT LEAST ONCE A YEAR EACH OF THE 26 LABS IS AUDITED BY LABORATORIO STANDARD LATTE. THE AUDITOR ASSURES THAT THE PROCEDURES ARE FOLLOWED CORRECTLY

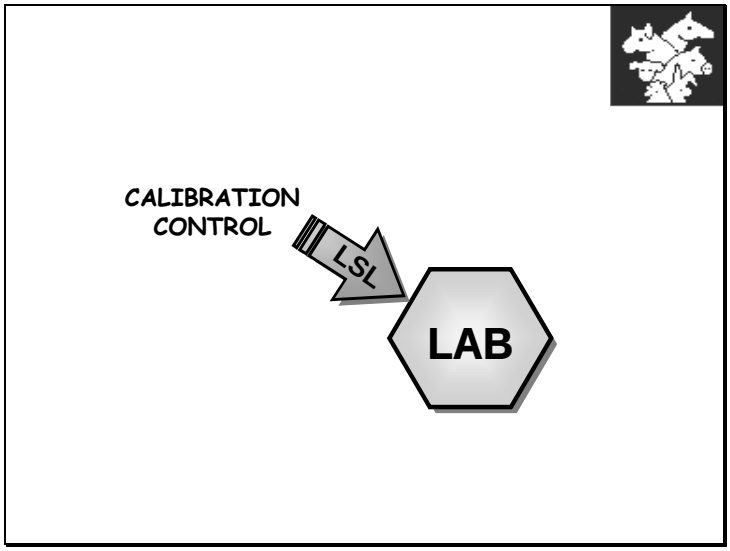
CONTROL

↓

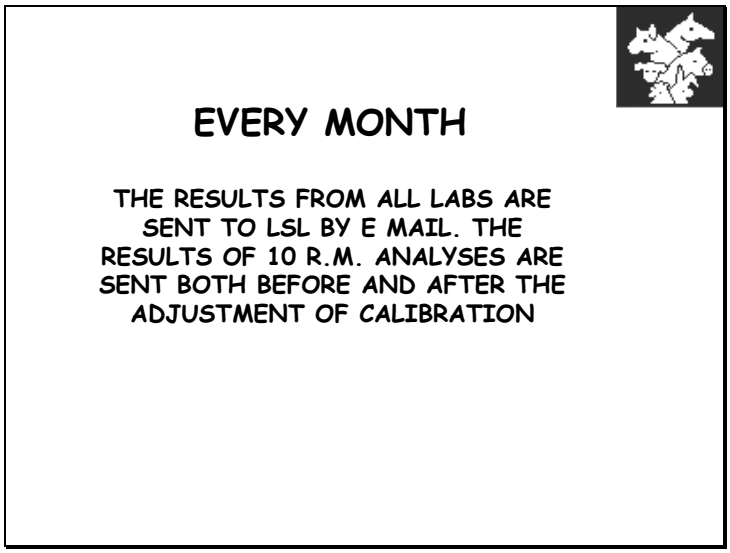
Dia 18



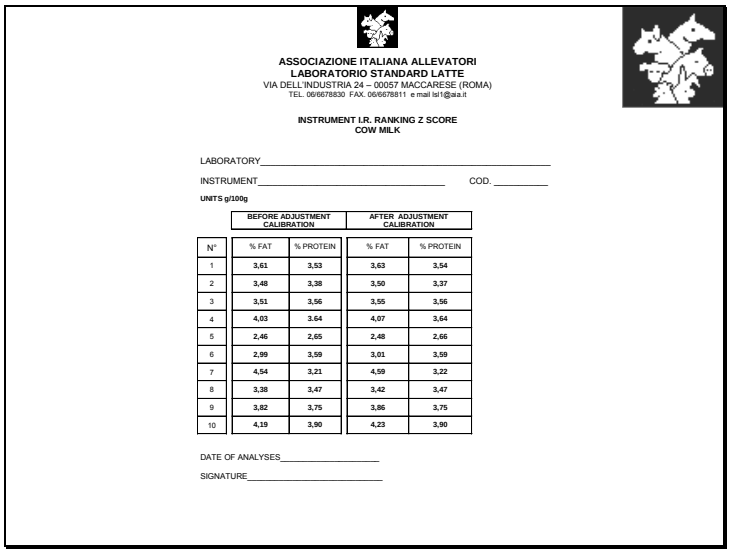
Dia 19



Dia 20



Dia 21



ASSOCIAZIONE ITALIANA ALLEVATORI
LABORATORIO STANDARD LATTE
VIA DELL'INDUSTRIA 24 – 00057 MACCARESE (ROMA)
TEL. 06/6678830 FAX. 06/6678811 e mail lsl1@aia.it

INSTRUMENT I.R. RANKING Z SCORE
COW MILK

LABORATORY_____

INSTRUMENT_____ COD. _____

UNITS g/100g

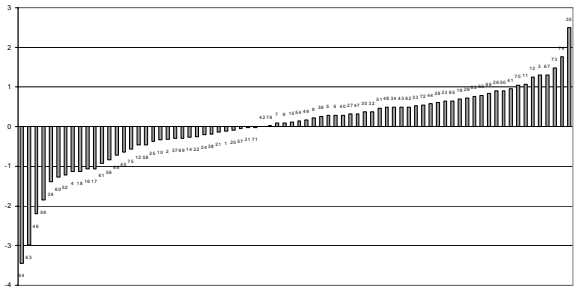
	BEFORE ADJUSTMENT CALIBRATION		AFTER ADJUSTMENT CALIBRATION	
N°	% FAT	% PROTEIN	% FAT	% PROTEIN
1	3,61	3,53	3,63	3,54
2	3,48	3,38	3,50	3,37
3	3,51	3,56	3,55	3,56
4	4,03	3,64	4,07	3,64
5	2,46	2,65	2,48	2,66
6	2,99	3,59	3,01	3,59
7	4,54	3,21	4,59	3,22
8	3,38	3,47	3,42	3,47
9	3,82	3,75	3,86	3,75
10	4,19	3,90	4,23	3,90

DATE OF ANALYSES_____

SIGNATURE_____

Dia 22

I.R INSTRUMENT RANKING - Z SCORE APRIL 2002
FAT CONTENT



Dia 23

YEAR 2001

LSL CALCULATES THE Z SCORE

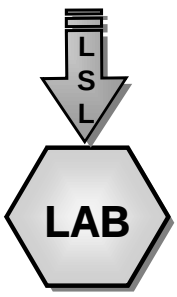
FAT 286 ELABORATIONS 19 LABS ZS>2 AND
3 LABS ZS>3

PROTEIN 286 ELABORATIONS 15 LABS ZS>2 AND
6 LABS ZS>3

RESULTS ARE COMMUNICATED WITHIN 5 WORKING DAYS

Dia 24

CALIBRATION AND REAPETABILITY CONTROL



Dia 25

CALIBRATION AND REPEATABILITY CONTROL

QUATERLY ALL LABS RECEIVE 12 COW MILK SAMPLES

THE LABS CARRY OUT FAT, PROTEIN, LACTOSE AND SOMATIC CELL COUNT TWO TIMES AND SEND BY E MAIL THE RESULTS TO LSL

Dia 26

CALIBRATION AND REPEATABILITY CONTROL

NOVEMBER 2001

FAT

	I RIP	II RIP	Diff	Media	Rif	Dif(M - R)
1	4,59	4,59	0,00	4,590	4,564	0,026
2	4,04	4,02	0,02	4,030	4,040	-0,010
3	2,44	2,44	0,00	2,440	2,439	0,001
4	3,47	3,47	0,00	3,470	3,452	0,018
5	4,76	4,75	0,01	4,755	4,764	-0,009
6	3,56	3,56	0,00	3,560	3,574	-0,014
7	4,24	4,25	-0,01	4,245	4,219	0,026
8	4,23	4,25	-0,02	4,240	4,216	0,024
9	3,12	3,13	-0,01	3,125	3,089	0,036
10	3,57	3,57	0,00	3,570	3,571	-0,001
11	3,60	3,61	-0,01	3,605	3,568	0,037
12	2,68	2,68	0,00	2,680	2,642	0,038
			EM	0,014		
			DSD	0,019		
			DSR	0,010		

PROTEIN

	I RIP	II RIP	Diff	Media	Rif	Dif(M - R)
1	3,35	3,33	0,02	3,340	3,302	0,038
2	3,62	3,62	0,00	3,620	3,614	0,006
3	3,37	3,38	-0,01	3,375	3,382	-0,007
4	2,88	2,87	0,01	2,875	2,863	0,012
5	4,02	4,02	0,00	4,020	4,018	0,002
6	3,24	3,25	-0,01	3,245	3,236	0,009
7	3,15	3,16	-0,01	3,155	3,137	0,018
8	3,15	3,15	0,00	3,150	3,136	0,014
9	2,96	2,96	0,00	2,960	2,963	-0,003
10	3,25	3,25	0,00	3,250	3,233	0,017
11	3,23	3,24	-0,01	3,235	3,203	0,032
12	3,24	3,25	-0,01	3,245	3,228	0,017
			EM	0,013		
			DSD	0,012		
			DSR	0,009		

Dia 27

YEAR 2001

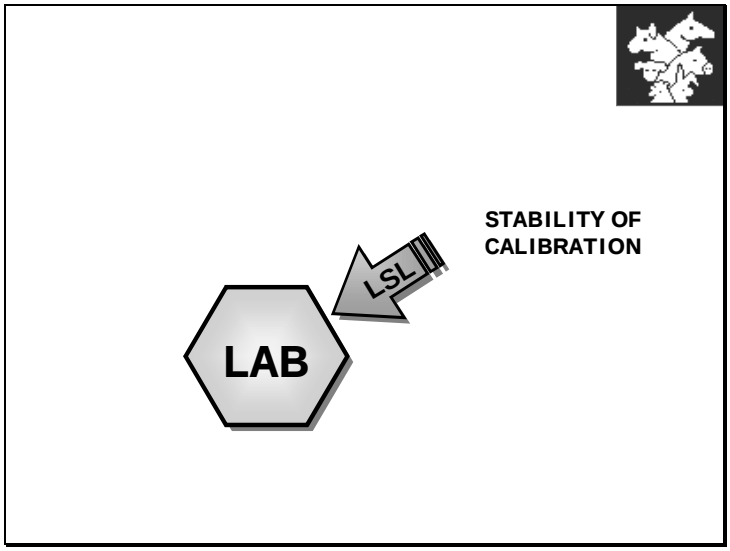
NO LABORATORIES SURPASSED THE LIMITS OF:

· STANDARD DEVIATION OF DIFFERENCE: 0,1 (FIL/IDF 141 C: 2000)

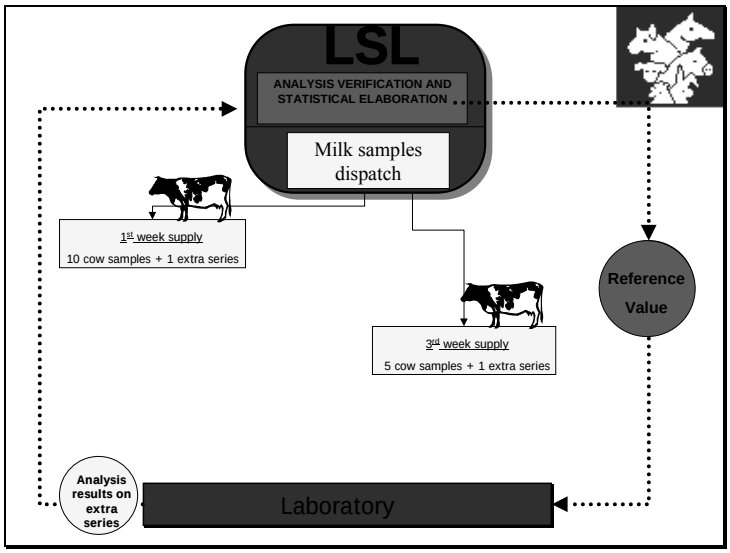
·ALGEBRIC DIFFERENCE: 0,06 g/100 g (FIL/IDF 141 C: 2000)

RESULTS ARE COMMUNICATED WITHIN 5 WORKING DAYS

Dia 28



Dia 29



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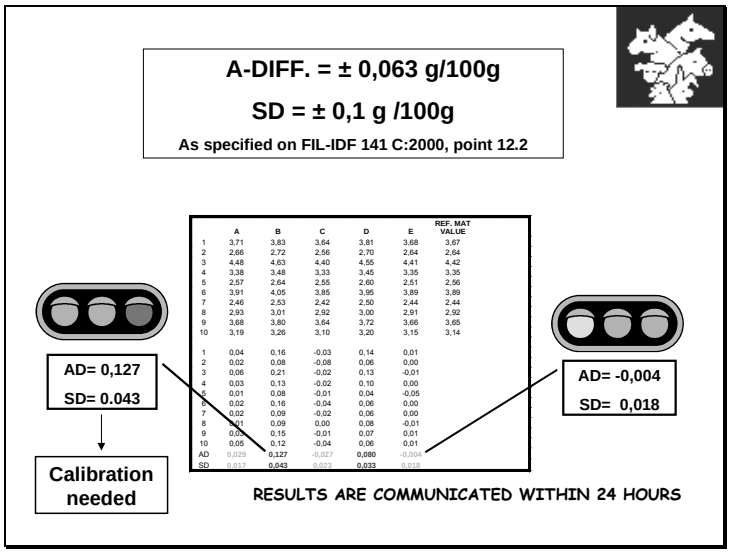


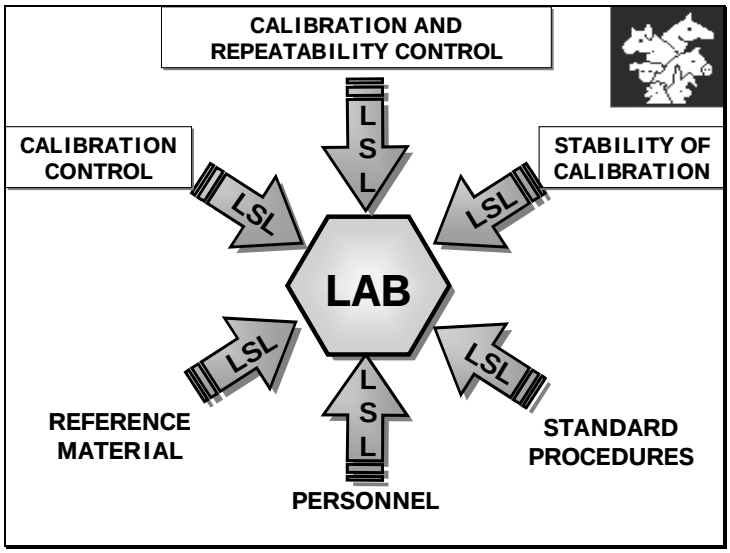
Dia 31

RESULTS 26 LABS

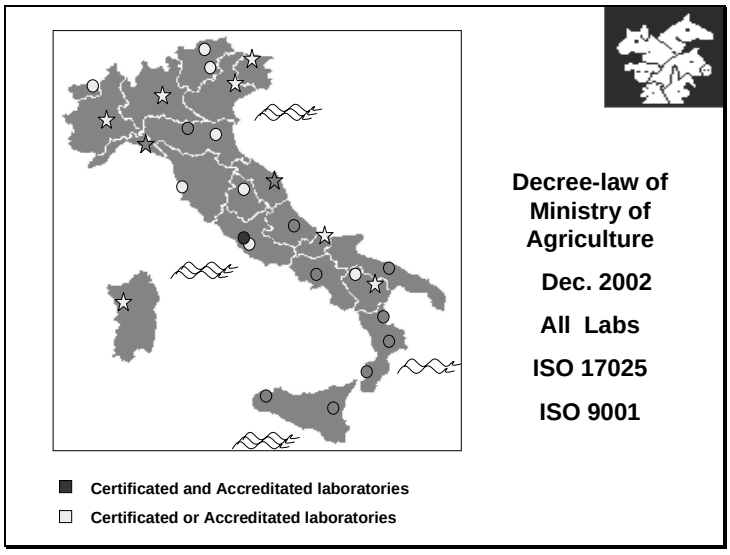
(Year 2002)

MONTH	10 R.M.		5 R.M.	
	FAT	PROT	FAT	PROT
JAN	2	0	3	2
FEB	2	1	1	0
MAR	1	1	0	0
APR	2	2	0	0
MAY	3	4	0	0

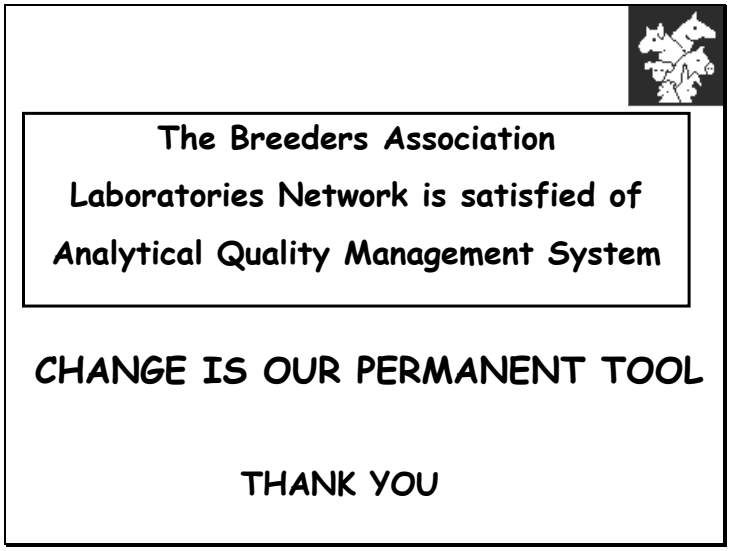
Dia 32



Dia 33



Dia 34


The Breeders Association
Laboratories Network is satisfied of
Analytical Quality Management System
CHANGE IS OUR PERMANENT TOOL
THANK YOU

Analytical Laboratory System and its QA/QC systems in the United States

John Rhoads
Eastern Laboratory Services, Fairlawn, Ohio (US)

The Council for Dairy Cattle Breeding (CDCB) governs the milk recording business in the United States. They employ National Dairy Herd Improvement Association (NDHIA) to implement the guidelines set forth by the CDCB. NDHIA has a subsidiary called Quality Certification Services that carries out and monitors all QA\QC guidelines in relation to labs, meters and field service.

The Federal Milk Market Administrator monitors payment testing. The Federal Milk Market Administrator is a government agency that establishes all the requirements for milk payment laboratories. Some milk recording labs are also payment laboratories. There are 42 payment only laboratories in the United States.

The geography covered by the dairy industry in the United States is vast covering 9,629,091 km2. 48 milk-recording laboratories scattered geographically and based on cow population's cover the region. 4,226,692 cows or approximately 50% of the cows are enrolled in milk recording. Avg. Fat test 3.66%, true protein 3.08%. and milk 20,846 milk lbs.

All laboratories are certified by Quality Certification Services on a yearly basis. Each lab is required to participate in monthly proficiency testing. 12 blind samples in duplicate are sent to each lab monthly for Fat, True protein, OSCC and MUN analysis. Sample results from each lab are entered into a web site (www.QCS.com) and results for each lab are compiled and generated. Labs that do not meet established guidelines are required to take corrective action immediately. All laboratories must pass a required on site audit of lab technicians, records and facilities on a biannual basis.

Dia 1

Dairy Quality Certification Services

John Rhoads
Manager of Laboratories
Eastern Laboratory Services

Dia 2

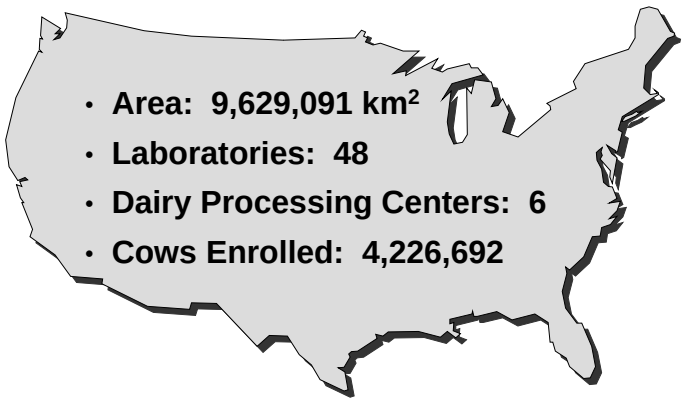
Council for Dairy Cattle Breeding

National Dairy Herd Improvement Association

Quality Certification Services

Dia 3

United States of America



- **Area: 9,629,091 km^2**
- **Laboratories: 48**
- **Dairy Processing Centers: 6**
- **Cows Enrolled: 4,226,692**

Dia 4

Laboratory Locations

Dia 5

Laboratory Audits & Certification

Dia 6

Initial Certification Audits

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

Dia 7

On-site Audits

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

Dia 8

Monthly Audits of the Sample Unknowns

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

Dia 9

Auditing of Infrared and Oscc Instruments for Sample Unknowns

Calibration Check Procedure

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

Acceptable Readings for Calibration Checks IR

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

Acceptable Readings for OSCC

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

Dia 10

Verification of Documentation

- At the Discretion of the Auditor, Individual Training Records May Be Reviewed or Interviews Held With Laboratory Technicians to Audit the Training Program in Place.

Dia 11

Auditing of Calibration Check and Maintenance Documentation

Calibration Checks and Maintenance Documentation

- All calibration checks and equipment maintenance records must be documented and provided during an audit.

Record Keeping Systems

- Calibration checks and maintenance records may be documented in the form of a computerized spreadsheet, manual listing, or other organized system. If manual listings are used, results should be recorded in ink.

- **Retention of Calibration Checks and Maintenance Documentation**
  - Documentation of all calibration checks and maintenance records should be maintained for a minimum of one year.

Dia 12

Auditing of Infrared Instruments

- **Calibration Adjustments**
- **Homogenization Efficiency**
- **Purging Efficiency**
- **Repeatability**
- **Zero Drift**
- **Pilot Samples**

Dia 13

Auditing of SCC Instruments

- **Calibration Checks**
- **Calibration Adjustments**
- **Pilot Samples**
- **Repeatability**
- **Zero Drift**

Dia 14

Analytical Laboratory System and its QA/AC System in the Czech Milk Recording

Oto Hanuš, J. Pytloun, J. Říha, P. Hering, E. Matouš, V. Genèurová
Research institute for cattle breeding, Ltd., Rapotín, (CZ)
Czech-Moravia Breeders Association, s.c., Prague (CZ)

Czech dairy cows milk recording (introduction)

In the milk recording (MR; with mark A4) of the Czech Republic (CR) 458,6 thousand of dairy cows are involved in 2002. It means 97,8 % in comparison to the total count of dairy cows (DC), being 468,5 thousand. The average milk yield of dairy cows in CR milk recording were 6136 kg of milk per lactation in 2001 (Tab. 1). The average milk composition and the milk components yield in CR milk recording are shown in Tab. 1, too. A certain worsening of the dairy cows reproductive parameters (increasing of service period about 18 days (18,2 %)) was observed unfortunately at the same time with distinct increasing of the milk yield about 33 % in the course of last ten years. In consideration of this fact there are clear practical work aims for next period.

The milk samples are taken by the workers of so called authorized breeding organizations on the basis of corresponded treaties in the framework of CR milk recording. Czech-Moravia breeders association (CMBA, Prague) is share company with property taking part of state, which carries out the milk recording and central following up of domestic animals according to corresponding law in the Czech Republic. This organization practises five routine milk laboratories (Tab. 2) for the individual milk samples analyses in the framework of ensuring and carrying out of the mentioned task in the Czech Republic.

In the framework of ensuring and controlling of the carried out service quality (QA/QC = quality assurance/quality control) the CMBA defended with the success the professional audit, which was carried out by the delegated ICAR workers (International Committee for Animal Recording) in 1994 year. On the basis of successful ICAR audit result the Czech milk recording (CMBA) obtained the right to using of the special prestige ICAR stamp on the written MR results materials (since 1996). It means the symbol of the quality guarantee of the CMBA work. In present time, only milk recordings of the Czech Republic, Slovenie and Slovak Republic have at ones disposal the mentioned right from the countries of the central Europe.

Laboratory system of Czech milk recording (material and methods)

There are the five routine milk testing laboratories (MTLs), which work under the little head of CMBA Prague in the framework of the Czech milk recording (Fig. 1). More details about the mentioned laboratories are shown in Tab. 2. The ratios between the total count of analyses about main milk components (fat (F), protein (P) and lactose (L) contents; 342 ths. per month) and the counts of analyses about somatic cell count (SCC) and urea (U) content are 51,8 and 2,9 % in the framework of dairy cows mammary gland health state and dairy cows nourishment state monitoring.

In general, it is very well known fact, that routine laboratories of the milk recording use especially indirect analytical methods for milk composition determination, which is necessary to check and calibrate regularly (2, 3, 4, 10, 11, 12, 13, 18, 27, 29, 35, 37, 39, 46, 48, 58, 60, 62, 74, 75, 76, 77, 78, 80, 81, 82, 85, 86). In the CR the mentioned fact still obtains for the fat, protein and lactose contents determination and partly for the somatic cell counts determination as well (16, 17, 21, 40, 51, 83). The original, specific and direct method UREAKVANT is used for the urea contents determination in the CR. This way fulfils all of assumptions of the reference method, according to our own results and in comparison to the results of other authors too (20, 34, 36, 45, 54, 59, 61).

In consideration of the above mentioned reasons, the MTLs are connected in networks with calibration/referential laboratories, which offer check and calibration (referential) materials, milk standards respectively, according to the scheme in Tab. 3 in regular time intervals. According to the same reasons, the MTLs are in connection with following check authorities in the Czech milk recording:

- for main milk components (F, P, L) checks and calibrations – testing accredited laboratory of the Research institute for cattle breeding (RICB), Ltd. in Rapotin, as a member of the official list of the ICAR-CECALAIT referential laboratory network;
- for checks and calibrations of somatic cell count determination in milk – testing accredited laboratory of the State veterinary institute in Prague and Bundesanstalt für Milchwirtschaft in Kiel (Germany);
- for checks and calibrations of milk urea content determination – Laboratory services Postřelmov and accredited milk testing laboratory of RICB Rapotin, as the member of the ICAR-CECALAIT referential laboratories proficiency testing (20, 36, 45, 54, 59, 61).

Quality system ensuring and controlling (QA/QC) of the work reliability in the MTLs network of dairy cows milk recording in the Czech Republic (results)

1. Routine MTLs in the dairy cows milk recording

The milk parameters determination in the Czech Republic is made according to schemes, which are shown in Tab. 4, 5 and 6. The connection of the referential and routine laboratories in calibrations and proficiency testing systems is in accordance with the scheme published by GRAPPIN (26, 28; Fig. 2). In this way the main premises of the organization for QA/QC system of milk testing laboratories (MTLs) in the Czech Republic milk recording are created.

A subsequent important factor the existence of the QA/QC system is the fact that the routine MTLs began with the process of accreditation. In 2001 some MTLs were accredited according to ČSN EN ISO/IEC 17025 standard, which has got an international validity, through the Czech Institute for Accreditation (CIA) Prague.

2. Referential laboratory in dairy cows milk recording

The accredited milk testing laboratory of the Research Institute for Cattle Breeding, Ltd., Rapotin (MTL-RICB) ensures the calibration activity (F, P and L milk contents), it means production of milk calibration (referential) standards and carrying out of interlaboratory proficiency testing (F, P, L and U milk contents) for system of routine MTLs in the dairy cows milk recording of the Czech Republic.

MTL-RICB has been a member of the official list within the ICAR-CECALAIT network of referential milk testing laboratories since 1996 (38, 43, 47, 49, 50, 52). This fact supports the QA/QC system of the above mentioned laboratory (MTL-RICB) in Rapotin. In this sense it is Jan Říha and Oto Hanuš who managerially bear the responsibility for MTL-RICB activities according to ICAR official member list. The MTL-RICB ensuring the set of the following activities for routine MTLs and plays role of:

A – national ring test organizer (32, 46);
B – reference material supplier (35, 36, 37, 39, 43, 45, 46, 48, 52);
C – master laboratory for centralized calibration (39, 43, 46, 48, 52);
D – training in laboratory techniques;
E – information provider about analytical methods (20, 21, 22, 23, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55);
F – subject for evaluation of analytical methods and instruments (references as in E);
G – subject for research of milk analytical methods (14, 20, 21, 22, 23, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 63, 69, 70, 72, 87);
H – subject for co-operation in the field of milk analytical methods development (30, 36, 45, 53, 54, 55);
I – subject for research of conditions of primary milk production, milk quality, milk hygiene, milk analyses and health state of dairy cows;
J – subject for advisory service (30, 33, 53, 55) concerning some troubles with raw milk quality in the primary production (direct advisory service towards farmers or with registration and financial support by state official agricultural authority).

Within the QA/QC system the MTL-RICB as a representative of the CMBA Prague takes part in ICAR-CECALAIT laboratory proficiency testing for the determination of the main milk components by referential methods (F, P, L, U) two times per year. The corresponding results had been published regularly (38, 43, 47, 49, 50, 52) and used with:

- QA/QC systems development for routine MTLs and for MTL-RICB;
- routine MTLs and referential MTL-RICB accreditations (estimations of validation parameters values and corresponding measurement uncertainties of milk analytical methods (19, 79));
- development of the systems design for statistical evaluation of ring tests results of milk analyses in dairy cows milk recording in the Czech Republic ((24, 25, 32, 64, 65, 66, 84) systems of evaluation of the so-called „Euclidian distance to the origin" in mutual comparison to the so-called „Z-score"results);
- co-operation in the development field of some new milk analytical methods (for instance UREAKVANT or KETOTEST (30, 36, 45, 53, 54, 55)).

MTL-RICB was accredited within the QA/QC system creation according to ČSN EN ISO/IEC 17025 standard (19) through CIA Prague during 2001 and 2002. Namely for 32 analytical methods (for milk, feedstuffs, drinking and waste water; for chemical, biochemical, physical and microbiological methods). The mentioned accreditation (concurrently corresponding with certification according to ISO 9002 (19)) has an international validity owing to CIA being full-right member and signatory of the following organizations:

- EA (European co-operation for Accreditation);
- ILAC (International Laboratory Accreditation);
- IAF (International Accreditation Forum).

Creation and utilization of the QA/QC system in milk laboratories of dairy cows milk recording in the Czech Republic (discussion)

1. The connection of the Czech milk recording laboratories with international proficiency testing

After connection of MTL-RICB into international interlaboratory co-operation in ICAR-CECALAIT network of referential laboratories it has been reached the significant progress in the field of quality management (QM) building in the system of Czech milk recording laboratories both referential and routine (28, 38, 43, 47, 49, 50, 52, 64). Finally, in comparison to previous period, the stable reliable basis for comparison possibility of results reliability exists periodically. It gives the possibilities of corrections in own methodical procedures in the case of necessity.

2. The networks of milk laboratories in the Czech Republic

In opposition to previously mentioned reached progress the shortcomings exist of course as well, for instance in particular the above mentioned separation of laboratory networks about milk quality determination for payment purposes (bulk milk samples) and for milk recording (individual milk samples). Introduced laboratory networks separation exist unfortunately as result of historical reasons both on the level referential and on the level of routine laboratories. This fact is not advantageous for analytical results reliability maximalization. It is very well known thing. Above introduced trouble should be solved in next future. Further laboratory concentration and laboratory network unification is not professional but political and legal problem because of existence of complicated property relationships. Most of laboratories are in some form of private property. Therefore is not possible to finish the activity any from these laboratories by some government legislative directive and so on. Objective process of laboratory variability reduction or more laboratory and analytical methods unification have to pass.

3. The referential methods for indirect methods calibrations

In the Czech milk recording used referential methods are the same as for milk pyment purposes according to its quality. They are used for indirect analytical methods calibrations in both of cases and are as follows:

T – acidobutyrometrical method according to Gerber (fat in milk in g/100ml (%)), ČSN 57 0530 (15);

B – mineralization-distilation-titratable method according to Kjeldahl (crude protein in milk; total nitrogen×6,38; in g/100g (%)), ČSN 57 0530 (3, 4, 13, 15, 29, 39);

L – polarimetrical method (lactose monohydrate in g/100g (%)) according to ČSN 57 0530 (15);

SCC – direct microscopical of SCC determination according to ČSN EN ISO 13366-1 (16).

The direct specific enzymatic method UREAKVANT is used for the routine determination of the milk urea content (it is characterized by the referential method parameters) in the Czech milk recording, which was developed in the Czech Republic (CR) as well (20, 30, 36, 45, 54, 59, 61).

The CR situation is similar to EU countries for cases of P, L and SCC. However extraction-gravimetrical method according to Röse-Gottlieb (fat in milk in g/100g (%)) is used as referential method for IR instrumental calibration in the case of F in the EU countries. Mentioned method is considered as referential in the CR as well, in particular for competent authority purposes (ČSN 57 0530 (15, 68)). Nevertheless, the method according to Gerber is in the validity as referential for indirect methods calibration purposes. We can expect the passage from Gerber´s method to Röse-Gottlieb´s method for indirect milk analytical method calibration purposes also in the Czech milk recording in the next future.

The Milko-Scan 133 B (Foss Electric, Denmark) is used in the role of IR master instrument for indirect analytical method in the MTLs system of Czech milk recording in the referential MTL-RICB Rapotin now.

4. The results uncertainty estimations in the Czech milk recording laboratories system

For QA/QC systems introducing and for legislative and administration purposes it was necessary to carry out the estimations of spreaded combined uncerntainty of measurement results (combined uncerntainty according to covariance law about uncerntainties spreading (79) ×1,96 for 95 % probability level) at analytical methods validation for accreditation in referential and routine milk laboratories of Czech milk recording. It all was made according to rule: „No accreditation without validation". Today used the results uncerntainties values are shown for the individual determined parameters (F, P, L, SCC, M) in Tab. 7. The results of the Czech milk laboratories taking part in interlaboratory analytical proficiency testing were used with advantage at these calculations in particular from the ICAR-CECALAIT testing (38, 43, 47, 49, 50, 52).

5. The results evaluation model in analytical proficiency testing in the Czech milk recording laboratories

The ICAR-CECALAIT model (28, 64) of the evaluation of the ring interlaboratory proficiency testing on the basis of synthetic parameter calculation (so called „Euclidian distance to the origin") was used at processing of the systems design of similar evaluations within our own network of routine milk laboratories in the framework of the Czech milk recording.

6. The Czech milk recording referential laboratory activities

In the framework of above mentioned points from A to E the referential laboratory (MTL-RICB) in Rapotin carries out the above introduced activities which are connected with practical existence supporting of QA/QC systems in the laboratory network of the Czech milk recording (32).

In the connection with point F, the evaluations row was carried out first of all in relationship to milk analytical methods, to factors influencing their calibrations and so on (21, 35, 36, 37, 39, 40, 41, 45, 48, 51, 54).

In the framework of point G, the one´s attention was given to milk samples transport conditions testing and their treatment by preservation (1, 5, 10, 22, 23, 31, 32, 41, 42, 44, 56, 67, 71, 73). Further, the microbiological methods for quantitative determination of occurrence frequency of main mastitis pathogens such as Staphylococcus aureus and Streptococcus agalactiae in the bulk milk samples were modified in specific way in the MTL-RICB in Rapotin. This methods are used by the laboratory of the State veterinary administration of the Czech Republic as governmental competent authority in this time (original papers 6, 7, 8, 9).

In the framework of H point there were carried out following co-operations:

- at the development of the specific enzymatic automatic method for milk urea content determination UREAKVANT, which is succesfully and routine used in the Czech milk recording laboratories (Tab. 2). The development was motivated by a lower reliability of milk urea content analytical methods with classical infrared (IR) technology instruments (without connected IR spectrum and Fourier´s transformation procedure (36, 54, 57)). It is original specifical methods with using of new reactor construction in which is fixed urease on inside surfaces. The electrical conductivity is measured before and after the specific enzymatic reaction in the modified milk sample (54, 59). The difference between two times measured conductivities, which is determined by the lytic reaction products rise, is calibrated according to known milk urea content. In the framework of method taking part in ICAR-CECALAIT testing, the possibility of impact of matrix changes in real milk samples and calibration standards on reliability of milk urea content determination results by mentioned method was studied (estimation of correction articles for evaluating software of the method UREAKVANT (30));
- at development of field price available test for fast semiquantitative determination of ketone (acetone) concentration in milk. It means for the farmer purposes at the cowhouse diagnostic and dairy cow health state monitoring – KETOTEST (53, 55).

In the framework of I point, some interpretation methods were developed on the basis of own and literature research results on the filds of primary milk production, milk quality and dairy cows health state:

1) prevalence estimation of intramammary Staphylococcus aureus and Streptococcus agalactiae infections in herds by examination of bulk milk samples. It is advantageous to useing this procedure in conditions of regular watching of mentioned pathogens occurrence in the bulk dairy cows milk samples (Tab. 8, Fig. 3 (8)).
2) occurrence state estimation of some productive diseases in the dairy cows herds (Fig. 4 (33)). The methods were developed into routine application software (UREAPROT and SOMPROT).

In the framework of J point, the advisory service was carried out by MTL-RICB Rapotin on the field of raw milk quality and troubles connected with milk quality. It was carried out in primary milk production directly on agricultural farms. The experiences and results obtained by this activity were published in following proceedings of contributions, including generally known knowledge and experience:

1) Inhibition substances in milk, 1994;
2) Hygiene in milk production, 1995;
3) Control of mastitis in milk production, 1996;
4) Management of dairy cows rearing, 1997;
5) Qualitative aspects of milk production, 1998;
6) Breeding, nutritional and technological aspects of milk production and quality, 2000;
7) New trends in organizational, technological and hygienical procedures of raw milk purchase in the context to EU conditions, I, 2001;
8) New trends in organizational, technological and hygienical procedures of raw milk purchase in the context to EU conditions, II, 2001;
9) Rearing and breeding of cattle for competitionable production, 2001.

The laboratory system tasks in the Czech milk recording (conclusion)

A significant progress has been reached in quality management (QM) development up to now. Nevertheless, it is very important to know about possible shortcomings and to continue in next development and improvement of activities and functions of the laboratory system in milk recording. The quality assurance of analytical work

(development of the QA/QC systems) is continual, neverended process in the fact. It is possible to expect following activities in the Czech milk recording in the next future:

- change of referential method for milk fat determination within the calibration of the indirect methods;
- further development and introducing of new analytical methods and analysed milk parameters – more services for milk recording and farmers (primary milk production);
- procedure improvement in analytical results interpretations in milk recording and at advisory service for dairy cows breeders;
- improvement of diagnostic parameters and statistical methods in evaluation of proficiency testing and analytical work quality (QA/QC systems);
- improvement of the preparation methods for referential materials;
- more international laboratory integration;
- laboratory concentration and unification;
- connecting the milk laboratories networks separated until now;
- in general, continual improvement of reproduction and milk yield of dairy cows and milk composition in the whole milk recording.

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Tab. 1 Milk yield of the dairy cows and basic composition of the individual milk samples during standard lactations in the Czech milk recording in 2002 (according to results of Czech-Moravia Breeders Association, Prague).

Breed	First lactation						Second and other lactations						All lactations					
	Frequency	Milk	Fat		Protein		Frequency	Milk	Fat		Protein		Frequency	Milk	Fat		Protein	
		kg	%	kg	%	kg		kg	%	kg	%	kg		kg	%	kg	%	kg
Bohemian Spotted cattle	51218	5044	4,26	215	3,44	173	126951	5840	4,20	246	3,40	199	178169	5612	4,22	237	3,41	191
Holstein	58436	6651	4,02	268	3,29	219	92960	7277	4,07	296	3,28	239	151396	7036	4,05	285	3,29	231
Czech Republic in total	129040	5778	4,13	239	3,35	194	262787	6311	4,15	262	3,35	211	391827	**6136**	**4,14**	**254**	**3,35**	**205**

Breed means the cows registered in the herd book; % fat = g/100ml; % protein = g/100g;

Tab. 2 Milk testing laboratories (MTLs) which work in the Czech milk recording system of the dairy cows (according to material of CMBA, Prague).

Localisation of MTL	Number of employees	Number of the milk samples analysed per month (ths.)			MTLs are equiped by instruments
		Fat, Protein, Lactose	Somatic cell count	Urea	
Buštěhrad	18	120	55	3,5	2 × Bentley 2000 + Somacount 3000 2 × Milko-Scan 255 1 × Combi Foss 250 1 × Ureakvant
Veselí nad Lužnicí	10	58	25	2,0	1 × Bentley 2000 + Somacount 3000 1 × Milko-Scan 255 1 × Milko-Scan 133 B 1 × Ureakvant
Plzeň	8	35	25	–	1 × Bentley 2000 + Somacount 3000 1 × Milko-Scan 255
Brno - Chrlice	11	61	40	1,0	1 × Bentley 2000 + Somacount 3000 1 × Combi Foss 250 1 × Milko-Scan 255 1 × Ureakvant
Přerov	10	68	32	3,5	1 × Bentley 2000 + Somacount 3000 2 × Milko-Scan 255 1 × Ureakvant
Czech Republic	**57**	**342**	**177**	**10**	

Tab. 3 The scheme of the basic check and referential activities at routine analyses of the individual milk samples in the Czech milk recording.

Milk parameter	Frequency of the calibrations	Number of the calibration standards	Frequency of the interlaboratory proficiency ring tests	Samples number in the test	Internal periodical * check in the laboratory	Number of samples in the test	Note
Content: fat (F) protein (P) lactose (L)	11 × per year[1]	10, each measured two-times	4 × per year[1] officially 7× per year as information in Czech Republic	10, measured 2×	pilot after max. 200 samples	2 measured 2×	[1] = calibration and ring test are at the same time
Somatic cell count (SCC)	4 × per year[2]	20, each measured four-times	4 × per year[2] in Czech Republic 1 × per year as international	20, measured 4×	pilot after max. 200 samples check, high and low SCC per day	2 measured 2× 2 measured 4×	[2] = ring test and calibration are at the same time
Urea content (U)	according to necessity, usually more-times per day	5	2 × per year in Czech Republic 2 × per year as international	10, measured 2×	1 check sample after 60 samples[3]	1 measured 2×	[3] = if it is not in accordance, it is necessary to carry out the calibration

* = results are compared to estimated limits of Shewarts control diagrams (IDF International Provisional Standard 141 A :1999);

Tab. 4 The used principles at measurement of the individual milk parameters in the MTLs of the Czech milk recording.

Milk parameter	Analytical method and equipment principle	Modification	Units of measurements	Parameter definition
Content: fat (F) protein (P) lactose (L)	infra-red analyse; Milko-Scan, Bentley	fact = optical filters, specific wave-lengths; FT technology in plan	g/100 ml (%) g/100 g (%) g/100g (%)	triglycerides in fat balls; * crude protein (total N × 6,38); monohydrate
Somatic cell count (SCC)	fluorooptoelectronical method; Fossomatic, Somacount	classical running disc; flow cytometry	ths./ml	cells of the white blood row with the nucleuses and epithelial cells in milk
Urea content (U)	enzymatical (ureolytical); Ureakvant	measurement of the conductivity changes	mg/100ml mmol/l	part of the non-protein nitrogen matters in the milk

* = it measures peptidical binding, conventional calibration is made according to the crude protein content;

Tab. 5 The diagnostical parameters and discrimination limits of the quality of the carried out control and check steps of the used method at routine laboratory milk analyses in the Czech milk recording.

Milk parameter	Repeatability	Calibration:		Proficiency testing:		Pilot – discrimination limit for differencies to basic referential values	
		variability of the differencies to the referential method	mean difference to the referential method	variability of the differencies to the referential values	mean difference to the referential value	check pilot samples (check within day)	work pilot samples (check within day and between days)
Content:							
fat (F)	≤ 0,02 % [1, 3]	≤ 0,06 % [1, 3]	± 0,02 % [3]	≤ 0,06 % [1, 3]	± 0,06 % [3]	± 0,05 % [3]	± 0,05 % [3]
protein (P)	≤ 0,02 % [1, 3]	≤ 0,06 % [1, 3]	± 0,02 % [3]	≤ 0,05 % [1, 3]	± 0,04 % [3]	± 0,05 % [3]	± 0,05 % [3]
lactose (L)	≤ 0,02 % [1, 3]	≤ 0,06 % [1, 3]	± 0,02 % [3]	≤ 0,05 % [1, 3]	± 0,04 % [3]	± 0,05 % [3]	± 0,05 % [3]
Somatic cell count (SCC)	L ≤ 5 % [2, 3] H ≤ 3 % [2, 3]	–	± 0,05 [3, 5]	–	± 0,05 [3, 5]	± 30 ths./ml [3]	according to declaration
Urea content [4] (U)	≤ 3 % [2, 3]	–	–	≤ 5 % [2, 3]	± 7 % [3]	± 6,7 % [3]	± 6,7 % [3]

[1]= standard deviation; [2]= variation coefficient; [3]=convention; [4]= method at the level of referential method; [5]= difference of regression equation slope to 1,0; L = low level of SCC ≤ 300 ths./ml; H = high level of SCC ≥ 1000 ths./ml;

Tab. 6 The usual parameters of the calibration standard sets in the Czech milk recording laboratories.

Milk parameter	Unit	Range	Mean ± standard deviation
F	g/100 ml (%)	2,00 – 6,00	4,10 ± 1,20
P	g/100 g (%)	2,70 – 3,70	3,35 ± 0,30
L	g/100 g (%)	4,00 – 5,10	4,90 ± 0,25
SCC	ths./ml	50 – 900	400 ± 300
U	mg/100 ml	12 - 60	36 ± 19

Tab. 7 The results of the estimations of the spreaded combined uncertainties of the measurement results (combined uncertainty × 1,96 for 95 % confidence level) of the milk parameters used in the today Czech milk recording.

Milk parameter	Referential methods		Routine methods	
	absolute	relative (%)	absolute	relative (%)
F	D; REL ± 0,036 %	± 0,89	I; ROL ± 0,101 %	± 2,77
P	D; REL ± 0,029 %	± 0,86	I; ROL ± 0,085 %	± 2,59
L	D; REL ± 0,042 %	± 0,85	I; ROL ± 0,115 %	± 2,37
SCC	–	–	ND; ROL ± 14,3 ths./ml	± 9,30
U	D; ROL ± 2,20 mg/100 ml	± 8,31	D; ROL ± 2,20 mg/100 ml	± 8,31

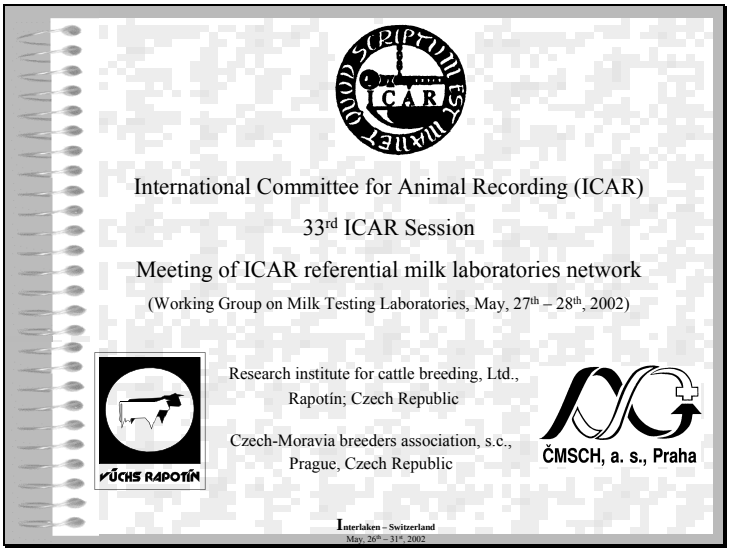
D = direct method; I = indirect method; ND = near direct method; REL = referential laboratory; ROL = routine laboratory;

Tab. 8 The prevalence estimation of the main mastitis ethiologies in the dairy cow herd according to frequency of the occurrence of the pathogens in bulk milk (according to BENDA et al., 1997).

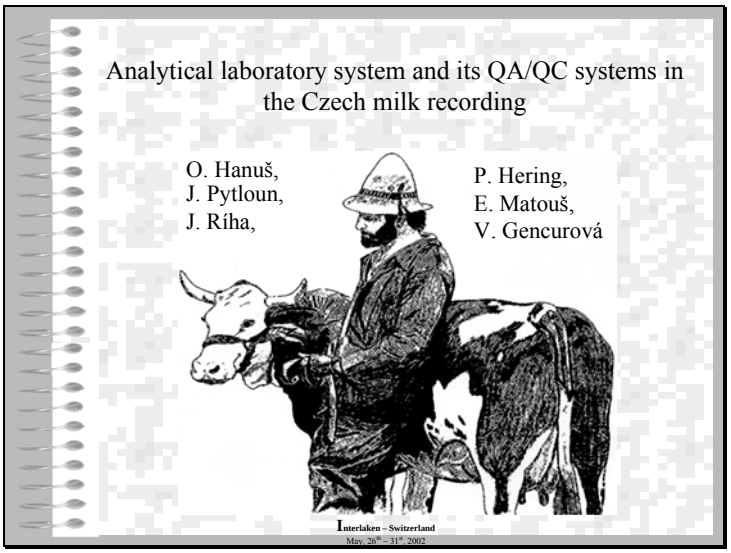
x value CFU/ml	*Staphylococcus aureus*			*Streptococcus agalactiae*		
	y estimation %	confidence level (90%)		y estimation %	confidence level (90%)	
0	**0**	0	3	**0**	0	1,6
10	**1,7**	0	8	**1**	0	4,5
50	**7,3**	0	17,7	**4,6**	0,1	10,4
100 *	**12,6**	2,1	26	**7,9 ***	1,8	15,7
200 **	**20,5 ****	5,7	38,4	**13,4**	4,8	23,7
300	**26,7**	8,5	47,8	**17,8**	7,2	30
500	**36,2**	12,7	62,6	**24,6**	10,9	39,9
700	**43,5**	15,8	74,2	**30**	13,8	47,8
1000	**52,1**	19	88,3	**36,4**	17,1	57,4
1500	**62,9**	22,3	100	**44,6**	20,9	69,9

* and ** are the convential levels which are significant for a high risk of the mastitis spreading in the herd;

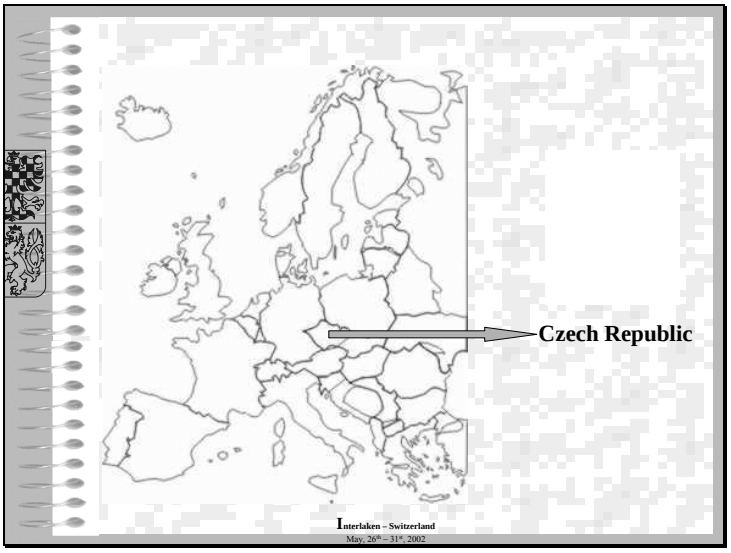
Dia 1



Dia 2



Dia 3



Dia 4

Milk yield of the dairy cows and basic composition of the individual milk samples during standard lactations in the Czech milk recording in 2002 (according to results of Czech-Moravia Breeders Association, Prague).

Breed	All lactations					
	Frequency	Milk	Fat		Protein	
		kg	%	kg	%	kg
Bohemian Spotted cattle	178169	5612	4,22	237	3,41	191
Holstein	151396	7036	4,05	285	3,29	231
Czech Republic in total	391827	**6136**	**4,14**	**254**	**3,35**	**205**

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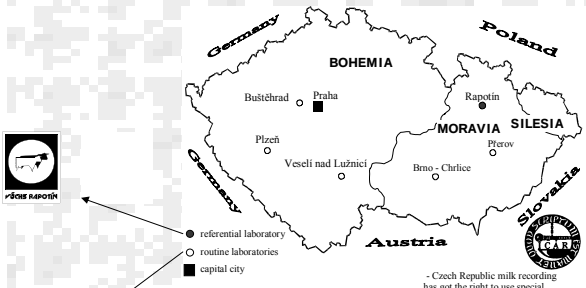
Dia 5

Laboratory system of Czech milk recording in the central Europe in 2002



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Dia 6

Milk testing laboratories (MTLs) which work in the Czech milk recording system of the dairy cows (according to material of CMBA, Prague).

Localisation of MTL	Number of the milk samples analysed per month (ths.)			MTLs are equiped by instruments
	Fat, Protein, Lactose	Somatic cell count	Urea	
Buštehrad	120	55	3,5	2 × Bentley 2000 + Somacount 3000 2 × Milko-Scan 255 1 × Combi Foss 250 1 × Ureakvant
Veselí nad Lužnicí	58	25	2,0	1 × Bentley 2000 + Somacount 3000 1 × Milko-Scan 255 1 × Milko-Scan 133 B 1 × Ureakvant
Plzen	35	25	–	1 × Bentley 2000 + Somacount 3000 1 × Milko-Scan 255
Brno - Chrlice	61	40	1,0	1 × Bentley 2000 + Somacount 3000 1 × Combi Foss 250 1 × Milko-Scan 255 1 × Ureakvant
Prerov	68	32	3,5	1 × Bentley 2000 + Somacount 3000 2 × Milko -Scan 255 1 × Ureakvant
Czech Republic	**342**	**177**	**10**	

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Dia 7

Development of the quality assurance/quality control (QA/QC) system in the Czech milk recording laboratories (MTLs):

- previous unofficial period (beginning of the QA/QC system, which existed at voluntary level without confirmation by a competent authority);
- official period:
  - ICAR audit in 1994;
  - ICAR prestige stamp using since 1996;
  - accreditation of the referential laboratory and some of the routine MTLs according to international standard ČSN EN ISO/IEC 17025. It consist mainly of:
    - quality book (quality system);
    - standard operation procedures of analytical methods;
    - validation of analytical methods.

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Dia 8

ICAR-CECALAIT proficiency testing as useful basis for the accuracy comparisons and control:

- the referential milk testing laboratory of Research Institute for Cattle Breeding in Rapotín (MTL-RICB) is member of the ICAR official list of the referential laboratory network since 1996;
- the MTL-RICB takes part in the ICAR-CECALAIT proficiency testing regularly;
- since this time the most probable true basis for result comparisons and control exists in the Czech milk recording labotarory system.

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Dia 9

The scheme of the basic check and referential activities at routine analyses of the individual milk samples in the Czech milk recording.

Milk parameter	Frequency of the calibrations	Frequency of the interlaboratory proficiency ring tests	Internal periodical check in the laboratory
Content: fat (F) protein (P) lactose (L)	11 × per year	4 × per year officially 7× per year as information in Czech Republic	pilot after max. 200 samples
Somatic cell count (SCC)	4 × per year	4 × per year in Czech Republic 1 × per year as international	pilot after max. 200 samples check, high and low SCC per day
Urea content (U)	according to necessity, usually more-times per day	2 × per year in Czech Republic 2 × per year as international	1 check sample after 60 samples

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Dia 10

The usual parameters of the calibration standard sets in the Czech milk recording laboratories.

Milk parameter	Unit	Range	Mean ± standard deviation
F	g/100 ml (%)	2,00 – 6,00	4,10 ± 1,20
P	g/100 g (%)	2,70 – 3,70	3,35 ± 0,30
L	g/100 g (%)	4,00 – 5,10	4,90 ± 0,25
SCC	ths./ml	50 – 900	400 ± 300
U	mg/100 ml	12 - 60	36 ± 19

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Dia 11

The diagnostical parameters and discrimination limits of the quality of the carried out control and check steps of the used method at routine laboratory milk analyses in the Czech milk recording .

Milk parameter		Repeatability	Calibration: variability of the differencies to the referential method	Calibration: mean difference to the referential method
Content:	fat (F)	≤ 0,02 % [1, 3]	≤ 0,06 % [1, 3]	± 0,02 % [3]
	protein (P)	≤ 0,02 % [1, 3]	≤ 0,06 % [1, 3]	± 0,02 % [3]
	lactose (L)	≤ 0,02 % [1, 3]	≤ 0,06 % [1, 3]	± 0,02 % [3]
Somatic cell count (SCC)		L ≤ 5 % [2, 3] H ≤ 3 % [2, 3]	–	± 0,05 [3, 5]
Urea content [4] (U)		≤ 3 % [2, 3]	–	–

[1]= standard deviation; [2]= variation coefficient; [3]=convention;
[4]= method at the level of referential method; [5]= difference of regression equation slope to 1,0;
L = low level of SCC ≤ 300 ths./ml; H = high level of SCC ≥ 1000 ths./ml;

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Dia 12

On the basis of the ICAR-CECALAIT proficiency testing results are carried out:

- regular check and control of referential results accuracy (running support of the QA/QC system);
- basic support of the QA/QC system:
  - accreditation:
    - validation parameter estimations;
    - result uncertainty estimations;
  - support of the content of the laboratory quality book;
  - support of the standard operation procedures of the milk analytical methods.

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Dia 13

The results of the estimations of the spreaded combined uncertainties of the measurement results (combined uncertainty × 1,96 for 95 % confidence level) of the milk parameters used in the today Czech milk recording.

Milk parameter	Referential methods		Routine methods	
	absolute	relative (%)	absolute	relative (%)
F	± 0,036 %	± 0,89	± 0,101 %	± 2,77
P	± 0,029 %	± 0,86	± 0,085 %	± 2,59
L	± 0,042 %	± 0,85	± 0,115 %	± 2,37
SCC	–	–	± 14,3 ths./ml	± 9,30
U	± 2,20 mg/100 ml	± 8,31	± 2,20 mg/100 ml	± 8,31

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Dia 14

Some of the referential milk testing laboratory (MTL-RICB in Rapotin) activities:

- accredited for 32 analytical methods:
  - raw milk, treated milk, drinking and waste water, air, feedstuffs;
  - chemical, biochemical, physical, microbiological methods;
- functions in MTLs system of the Czech milk recording:
  - centralized calibrations according to referential method results (F, P, L);
  - interlaboratory proficiency testing in the Czech Republic (F, P, L, U);
  - milk analytical method information and training;
  - co-operation on milk analytical method research and development, such as:
    - Ureakvant;
    - Ketotest;
  - primary milk production and dairy cow health state research:
    - mastitis state estimation method;
    - expert systems about dairy cow productive disorders;
  - advisory service for farmers towards higher milk quality, for instance by programmes too, such as:
    - Somprot;
    - Ureaprot.

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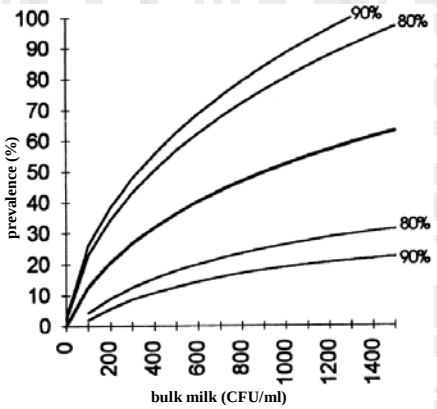
Dia 15

Prediction curve of *Staph. aureus* prevalence in herds, with confidence regions (by Benda et al., 1997)



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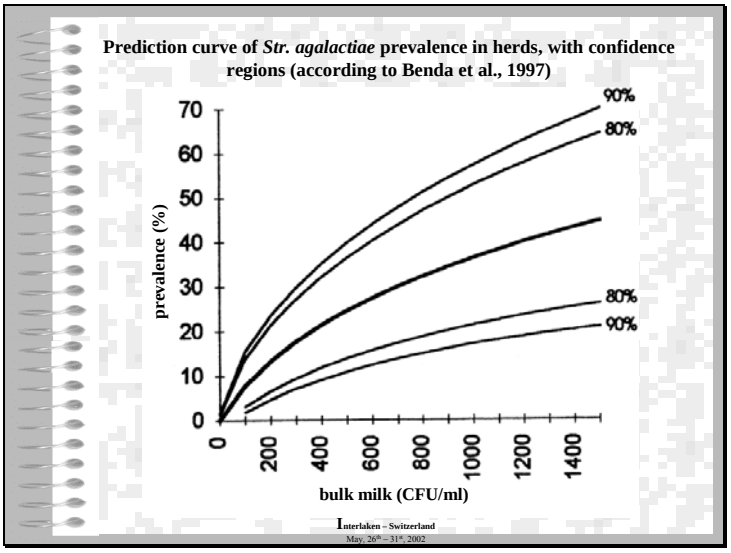
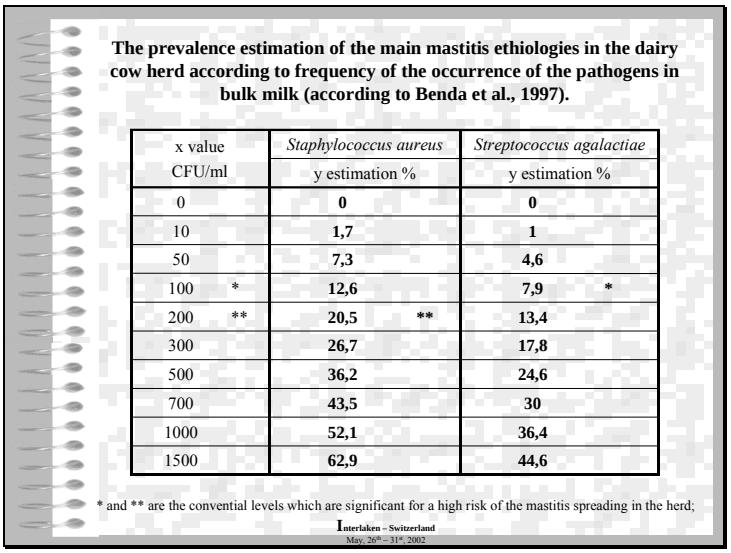
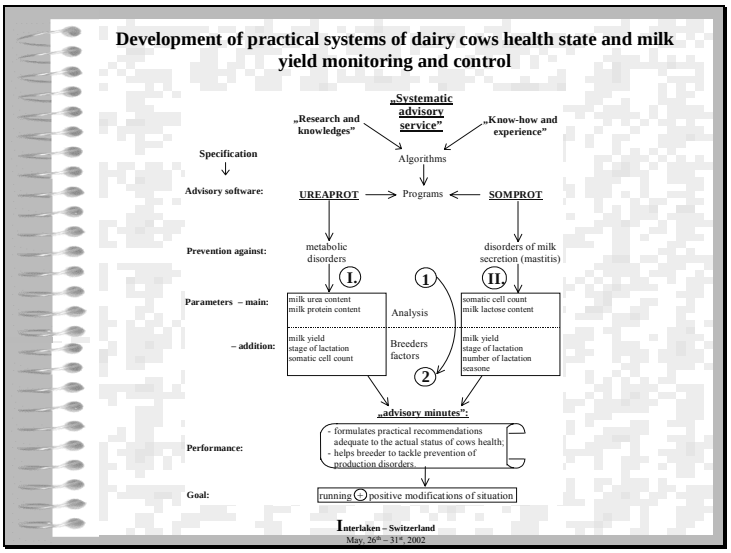
Dia 16



Dia 17



Dia 18



Dia 19

Disadvantages and tasks of the MTLs system of the Czech milk recording in the next future:

- to change Gerber´s referential method for fat content determination and calibration to Röse-Gottlieb´s method;
- unify and concentrate our historicaly separated milk testing laboratory system into two networks (I milk recording (5+1) and II milk payment (5+1) for both routine (10) and referential (2) laboratories as well) = very difficult task because of the fact, that all laboratories are private (however it is the necessity for the future accuracy and result reliability improvement);
- to do the running improvement of the QA/QC system in MTLs of the Czech milk recording. Of course, it is the neverended work.

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Dia 20

MLÉČNÁ A MIKROBIOLOGICKÁ LABORATOŘ
VÚCHS RAPOTÍN

„Thank You for Your kind attention"

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Evaluation on the Functioning of the ICAR Reference Laboratory Network

(notes from the discussion)
Harrie van den Bijgaart,
Netherlands Milk Control Station, Zutphen (NL)

General

The ICAR Reference Laboratory Network is expected to operate as:

- an international platform for diffusing Good Laboratory Practice (GLP) and Analytical Quality Assurance (AQA) based on international guides and standards to milk testing laboratories;
- the instrument to provide precision traceability and to promote international consensus in the outcome of analytical activities in dairy herd improvement analysis, this through the execution of International Proficiency Studies (IPS);
- means for promoting technical-analytical co-operation between laboratories.

Participants in the meeting indicated their appreciation for the functioning of the ICAR Reference Laboratory Network thusfar.

Communication

Communication is a major objective of the network as improvement comes from knowledge maintenance through regular information from various sources.

A frequent updating of the list of members with their competence is considered valuable.

It was noted that relevant documents, such as ISO/IDF standards and guidance on GLP and AQA, are generally obtained via other sources. The representatives from the network members are hardly familiar with more specific available guidance on AQA, (draft) protocols and reports, that are originating from the ICAR Working Group on Milk Testing Laboratories (MTL WG).

MTL WG has webspace available at www.icar.org. This can serve as an additional, permanently available, source of information.

Actions:

- *to provide updates of the list of members with their competence to the network members with a frequency of twice per year;*
- *to sent available guidance on analytical quality assurance and the draft protocol on the evaluation of milk analysers to network members;*
- *to upload information to the ICAR MTL WG webspace, such as:*

  MTL WG membership list;
  terms of reference;
  reports of the MTL WG meetings;
  date of the next MTL WG meeting;
  task underway;
  information on analytical methods for DHI;
  list of centres for testing analytical instruments;
  information on the ICAR Reference Laboratory Network.

Perception of the ring trials

The executed ring trials are very relevant, for instance with regard to the links needed for consensual values, precision traceability and the demands imposed by accreditating bodies. The results do serve as a regular check on the analytical performance and may for instance indicate problems with internal procedures, chemicals or equipment. It is stressed that conclusions should not be based on results from a single trial. Note should be taken, but it is only after consistency in results from two or three consecutive trials that measures are considered justified.

The presentation of the results was generally indicated as fit for use. A suggestion was made to provide more or better explanation on how to interpret the results and to avoid misjudgement by participating

laboratories and others. An overview on the development in the results from the ring trials could help in the explanation of the purpose and the promotion of network activities.

Action:

- *to consider possible improvement in the explanation on how to interpret trial results.*
- *to provide an overview in the development in precision and performance of participants during the years.*

Extension of activities

Within a few months it is intended to start with ring trials for sheep and goat milk. These will be organised by AIA – Laboratorio Standard Latte (Maccarese, IT). This initiative was very much welcomed by the network member representatives. Potential participants will be invited directly of through their National Member Bodies.

Action:

- *to seek participation in ring trials for sheep and goat milk.*

Evaluation of the meeting

The participants judged this first ICAR Reference Laboratory Network meeting as very fruitful, both for the absorption of information but even more for the personal contacts and the possibility to exchange experiences. Participants were very much in favour of organizing a follow-up meeting in two years from now during the next Biennial ICAR Sessions.

Action:

- *to plan for a Second ICAR Reference Laboratory Network meeting with the next ICAR Sessions in 2004.*