

Reference System for Somatic Cell Counting - On the Road to Better Global Equivalence -



IDF/ICAR Project
Reference System for Somatic Cell Counting

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- Analytical equivalence
- Relevance of somatic cell counting in milk
- Methods to count somatic cells in milk
- Present anchoring strategies in laboratories
- ICAR & IDF initiative in improving equivalence
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Drivers for analytical equivalence

When goods are moving,
analytical results need to be comparable

- Geographically
- In time
- Between different methods



Traceability is key for:

- A valid outcome of analytical work
- A uniform expression and use of results

Equivalence.....anywhere, anytime, anyway!!

Somatic cell count

Relevant in:

- Food legislation
- Payment of raw milk
- Farm management decisions and breeding programs

REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 29 April 2004
laying down specific hygiene rules for food of animal origin

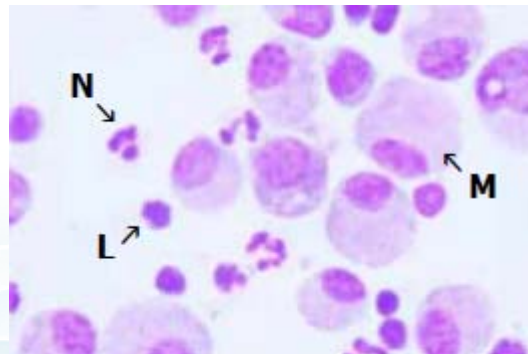
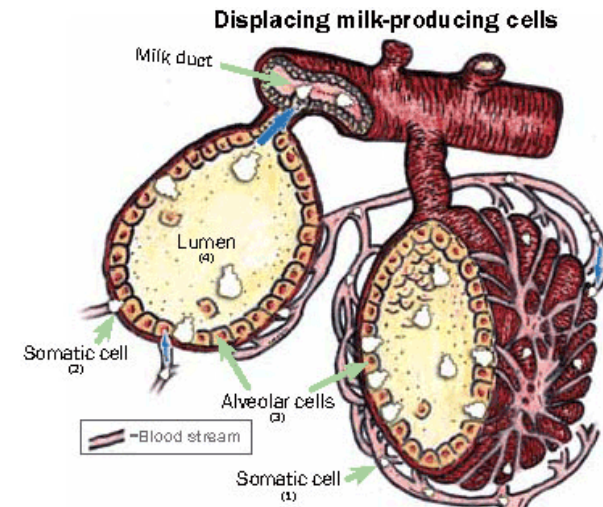


Millions of measurements per year in:

- Herd bulk milk samples
- Individual animal samples

Somatic cells

- Epithelial cells
- Leucocytes
 - Macrophages
 - Lymphocytes
 - Polymorphonuclear neutrophils
- Rapid increase in milk upon inflammation of the udder



Somatic cell counting methods

Defining method (DMSCC) (ISO 13366-1 | IDF 148-1)

- + Direct microscopic count
- Analyte poorly defined
- Laborious, cumbersome
- Requires well-trained analysts
- Poor precision



Routine methods (ISO 13366-2 | IDF 148-2)

- + Automated, user-friendly
- + High throughput
- + High precision
- Needs reference

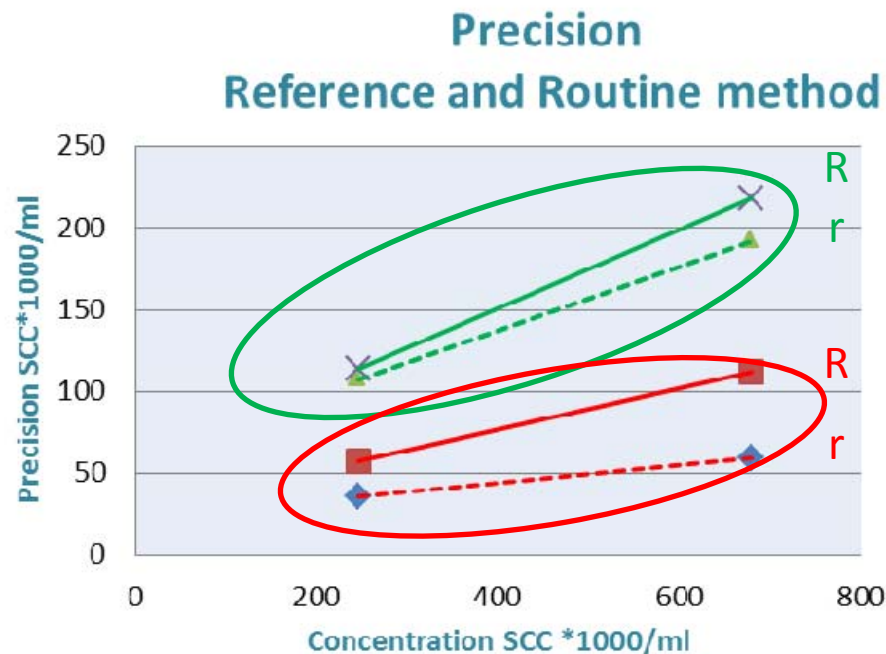


Precision DMSCC vs routine methods

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

All values in '000/mL

Source: ISO 13366-1/2 | IDF 148-1/2



Reference
Method

Routine
Method



Typical anchoring strategies used by labs

- Using Secondary Reference Materials (> 20 providers)
- Using in-house prepared SRM, characterized with reference method analysis....
....but also seeking a smoothening of variation by including routine method results
- Relying on the instrument manufacturer's settings and checking consistency with proficiency testing results
- Adapting based on the outcome of proficiency testing

Actually, SCC is a huge ship, sailing with many anchors!



Reference System for Somatic Cell Counting

IDF/ICAR initiative started in 2009.....

- To explore the feasibility of a reference system approach where traditional calibration schemes lack effectiveness
- Better safeguarding of worldwide equivalence of analytical test results, here for somatic cell counting



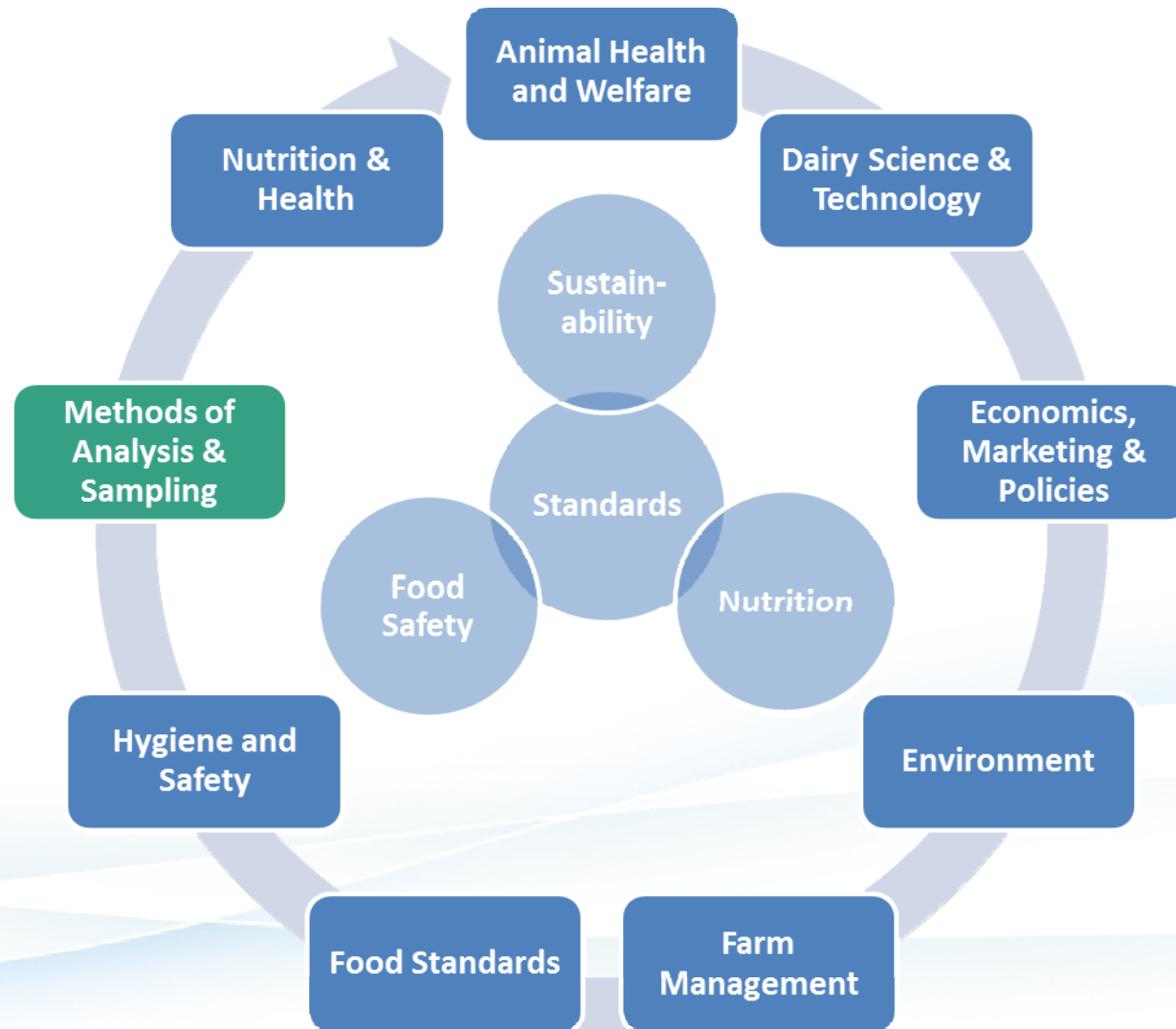
IDF mission



Helping to nourish the world with safe and sustainable dairy by providing science-based expertise and consensus for the global sector and be the global voice of dairy to intergovernmental organizations and stakeholders



IDF focus and related work areas



ICAR mission



Mission of ICAR is to be the leading global provider of Guidelines, Standards and Certification for animal identification, animal recording and animal evaluation. ICAR wants to improve the profitability, and sustainability of farm animal production by:

Establishing and maintaining guidelines and standards for best practice in all aspects of animal identification and recording.

Certifying equipment, and processes used in **animal identification, recording and genetic evaluations**.

Stimulating and leading: continuous improvement, innovation, research, knowledge development, and knowledge



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17 countries, 4 continents, RM and PT providers, regulatory agencies, research centers, dairy laboratories, instrument manufacturers!



Reference system

A well-structured anchoring system fed by different types of information (reference method results, routine method results, proficiency testing results) from sources with traceable competence, accepted by users and recognized/approved by competent authorities.

Bulletin of the International Dairy Federation 427/2008

Towards a Reference System for
Somatic Cell Counting in Milk

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

C. Baumgartner¹

Summary

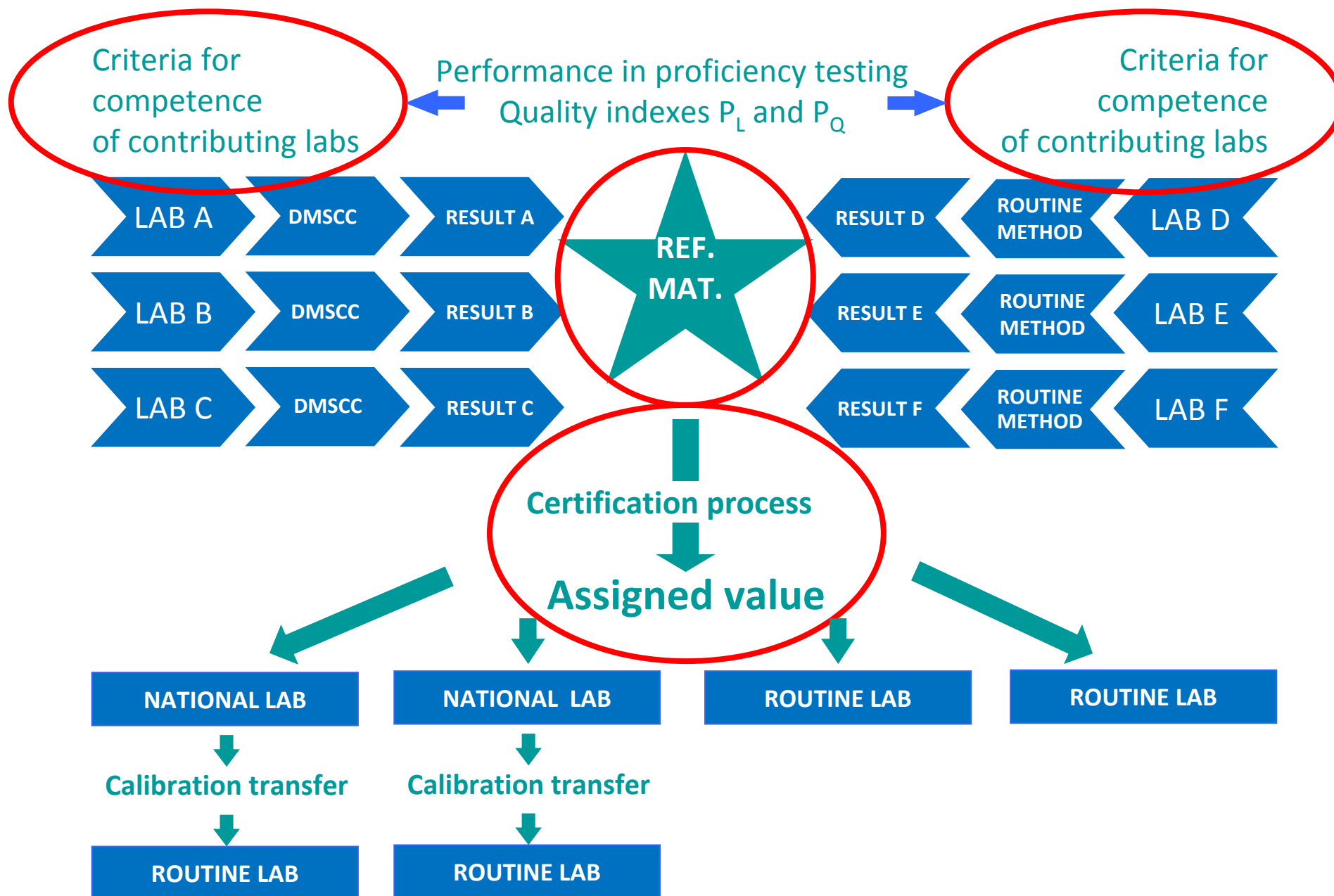
The definition of "reference" relates to two meanings. One relates to "testimonial", "endorsement" or "certification", the other to the aspect of "information", "evidence" or "source". These notions are a good description of the purpose of reference systems, which are intended to complement and improve the traditional way of calibration of routine methods.



Building blocks

- Motivation
- Skilled people
- Cooperation in a lab network system
- Reference documents
- Reference materials
 - Reference method results
 - Routine method results
- Proficiency testing
- Calculation model
- Communication





Achievements (1)

- Created much awareness about the project through publications, presentations, newsletters, website www.fil-idf.org/RSSCC
- 3 Questionnaires in 2010 to 2013
 - Reference material providers and organizers of proficiency testing
 - QA and anchoring in routine laboratories (214 replies)
 - Interest in certified reference materials (141 replies)
- Identified interlinkages between labs worldwide through use of secondary reference materials and participation in proficiency testing



Achievements (2)

Recent publications:

- Leray, O., Orlandini, S. & Braun, U. Requirements for Reference Materials for the Calibration of Automated Somatic Cell Counters. Bulletin of the IDF No. 469/2013.
- Di Marzo, L. Wojciechowski, K.L. & Barbano, D.M. 2016. Preparation and stability of milk somatic cell reference materials. J. Dairy Sci. 99 (9), pp 7679-7689.
- Berger, T. & Luginbühl, W. 2016. Probabilistic comparison and assessment of proficiency testing schemes and laboratories in the somatic cell count of raw milk. Accred. Qual. Assur. 21 (3), pp 175–183.

Liaison with ISO/TC 276 Biotechnology – Cell Counting:

- ISO/WD 20391 - Part 1 - General guidance on cell counting methods
- ISO/WD 20391 - Part 2 – Method to evaluate quality of a cell counting measurement process via experimental design and statistical analysis



Current activities (1)

Collaboration with EU JRC/F.Health, Consumers & Reference Materials – Geel :

- Preparation of certified reference material for somatic cell counting is on JRC work programme for 2015-2016
- Collaborative work started in 2014
 - Organizing access to raw material, processing, measurement needs
 - Familiarizing with preparation protocol
 - Test batches produced in December 2014 and February 2015



Current activities (2)

Collaboration with EU JRC/F.Health, Consumers & Reference Materials – Geel :

- Optimization of cell concentration process
 - Gravity separation
- Optimization of drying process
 - Freeze-drying
 - Spray-drying
- Commutability studies
 - Does the reference material behave like raw milk with different counting methods?
- Evaluating reconstitutability, homogeneity and stability
- Characterization of reference material
 - 15 labs qualified for reference method measurements
 - 20 labs qualified for flow cytometry measurements



Implementation and future functioning

Cooperation needs a structure!



- International organizations (animal performance recording, dairy)
- Regulatory supervisors
- Reference material providers
- PT organizers
- Pivot laboratories
- Routine laboratories
- Instrument manufacturers



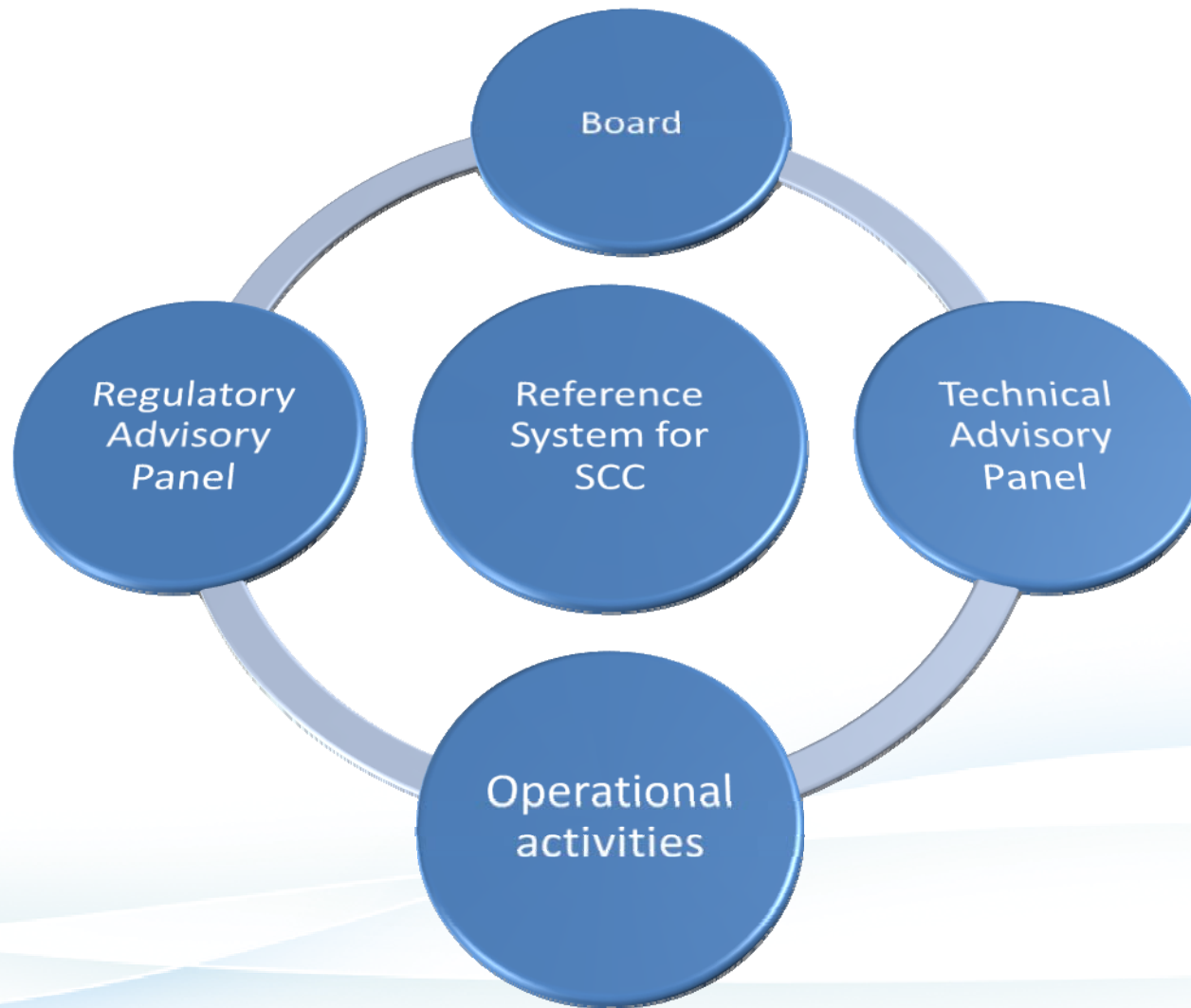
NeuBo mission (under construction)



Promoting and supporting optimal equivalence in analytical results for the dairy sector worldwide through the application of international standards and guidelines, reference materials, reference systems and participation in proficiency testing in an integrated, robust and neutral structure



NeuBo (under construction)



Proposed functions/roles for NeuBo

- **Implementation the functioning of the reference system for SCC** coordinating the characterization of RMs, monitoring and safeguarding the functioning of the reference system for SCC. This will include the creation and maintenance of a database with information provided by participants in confidentiality.
- **Communication**
 - **Communication** between participating pivot labs, regulatory supervisors and other stakeholders around SCC and for possible other parameters in future.
 - The **dissemination of information** on the why and the how of a reference system approach in addition to traditional calibration schemes, the provision of guidelines to stakeholders with regard to SCC and possible other parameters in future.
 - The **provision of** tailor made **advice and neutral consultancy services** to stakeholders.
 - Communicating the needs of the dairy sector for primary RMs and PTs in the proper places and **promoting their development and their standardized application.**



Proposed functions/roles for NeuBo (1)

Implementing the Reference System for SCC

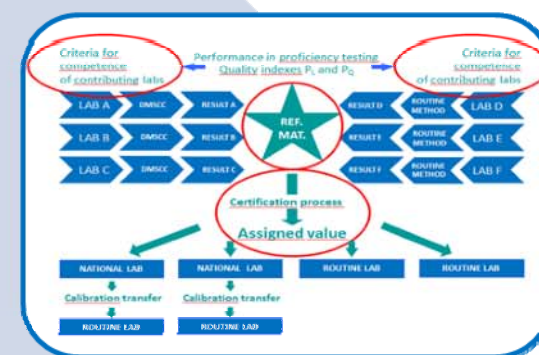
- Coordinating the characterization of RMs
- Monitoring and safeguarding the functioning of the reference system for SCC

This will include the creation and maintenance of a database with information provided by participants in confidentiality.

Database



PT information



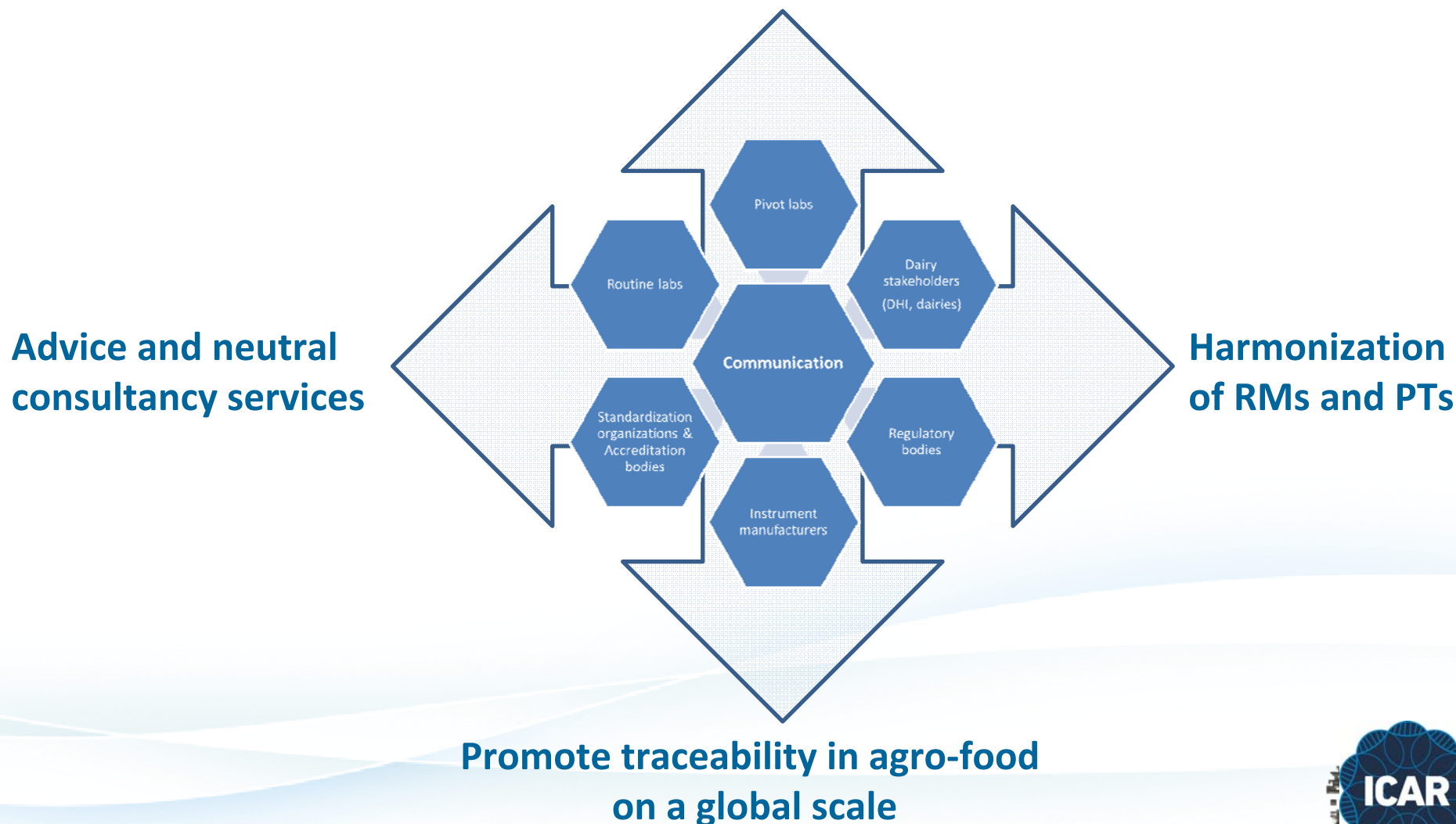
Proposed functions/roles for NeuBo (2)

Communication Platform



Communication platform will provide

Dissemination of information on RSSCC



Characteristics for NeuBo

- Neutral
 - Unbiased
 - Non-commercial
 - Solid stakeholder involvement
-
- Usefulness for other dairy analytical systems



Under discussion in the Project Group





Thank you for your attention!



Activities of EU JRC/IRMM (2)

- Some first results with different methods
(2-6 replicate measurements per sample)

	Raw milk	L milk after preparation BEFORE freeze-drying	H milk after preparation BEFORE freeze-drying
Direct microscopy CRA-W	130 ± 7	57 ± 4	989 ± 29
Image cytometry IRMM	127 ± 11	40 ± 3	820 ± 28
Flow cytometry MCC Lier	125 ± 10	46 ± 3 46 ± 5	900 ± 16 906 ± 22
Flow cytometry MPR Bayern	132 ± 3	44 ± 1	910 ± 24

Source: R Zeleny – JRC/IRMM, IDF/ISO Analytical Week 2015

Activities of EU JRC/IRMM (3)

- Some first results before and after freeze-drying with image cytometry at IRMM (3-4 replicate measurements per sample)

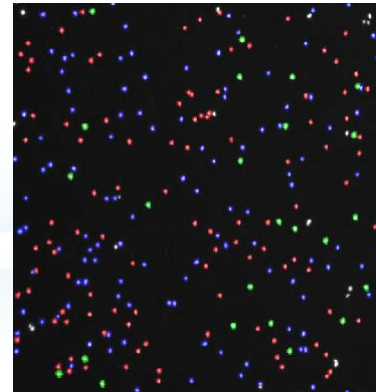
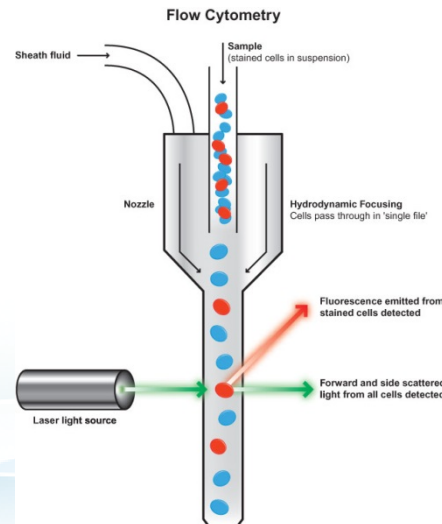
	milk BEFORE freeze-drying	milk AFTER AIA-LSL freeze-drying programme	milk AFTER IRMM freeze-drying programme
L	40 ± 3	43 ± 14 45 ± 5	46 ± 20 44 ± 3
H	820 ± 28	824 ± 34 879 ± 72	853 ± 40 852 ± 83

Source: R Zeleny – JRC/IRMM, IDF/ISO Analytical Week 2015

Current activities (3)

New work on the reference method:

- In the slipstream of work on differential somatic counting
- Automated microscopy
- Flow cytometry
- (Flow-based) Image cytometry



- Under consideration in IDF/ISO TC34 SC5

