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

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Abstract

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

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

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

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

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

Introduction

The parameter of somatic cell count in milk (SCC) is one of the most important indicators for udder health of cows and therefore for milk quality. More than 500 million analyses are estimated to be performed each year worldwide. This parameter is used in farm management. Breeding programs and it is a criterion for

hygiene regulation and milk payment. Due to the great importance of this parameter the ICAR/IDF project on "Reference System for Somatic Cell Counting" ("RefSysSCC") has the aim to enhance and secure analytical equivalence on a global scale. A fair international trade in the dairy sector requires that the results are comparable between laboratories, between countries and between methods used.

The analyses for somatic cell counting are performed worldwide using automated instruments. These instruments must be checked using appropriate quality assurance procedures and reference materials. The international procedures for reference and routine method were recently revised but reference method has a poor performance while certified reference materials are not available and the characterization processes of the secondary reference materials are not standardized. Due to the weakness of the quality assurance tools to achieve a better equivalence in somatic cell counting it is necessary to provide an international structure to anchor the analytical results: it will facilitate to built the analytical traceability. This is the most important goal of the project on Reference System for Somatic Cell. This project group is composed by 28 very active members from 19 countries and 4 continents! They agreed from the beginning to develop the project as to start from the present analytical situation and to consider the different analytical solutions adopted in the different countries by the laboratories. Each laboratory usually is a part of a group of laboratories that use the same quality assurance tools, e.g. the same secondary reference material or they participate in the same proficiency testing scheme or they use the same guidelines. For the project is extremely important to know "if and how" the different groups are connected.

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

Information gathered on reference material producers

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

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

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

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

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

Characteristics of reference materials for somatic celll counting	
Nature	Raw Milk UHT Milk Natural Cell External Cell from other matrices
Range	80.000-1.000.000 cells/ml 200.000-500.000 cells/ml 150.000-1.300.000 cells/ml
Number of samples /levels	2 4 5 10
Characterization processes	Proficiency Test with Ref.& Automated method Reference Method Automated and Reference Method
Shelf life	From 5 days to 180 days

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

Information gathered from routine laboratories

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

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

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

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

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

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

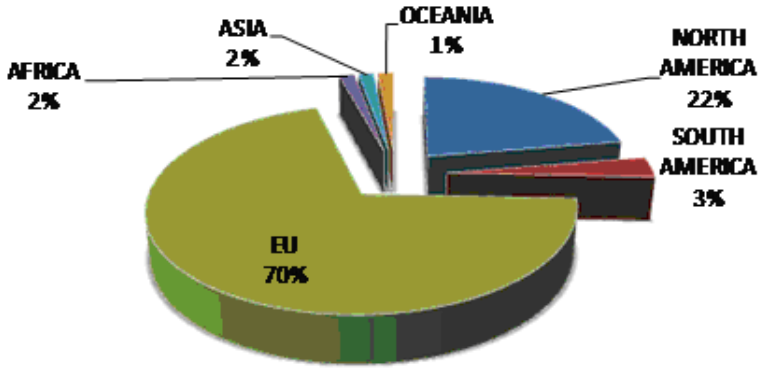

Figure 1. Geographical distribution of replies received.

We have registered 5 manufactures that produce instruments to determine somatic cell counting in milk and the technique used are: Flow cell (FC) 90%, displaying on disc (DD) 7% and charged coupled device (CCD) 3%.

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

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

The three main categories of reference material used are natural raw milk (64.25 %) heat treated milk (21.72) and skimmed/half skimmed (3.16%). 1 laboratory uses lyophilized milk, 1 frozen and 10 % don't use reference materials.

The synthetic particles 3% were not considered reference materials.

An other interesting information is that 9% of laboratories use either reference material with natural cells or synthetic particles. 12% of labs use reference material from different providers. This information is confirmed from the answer received for question 5.

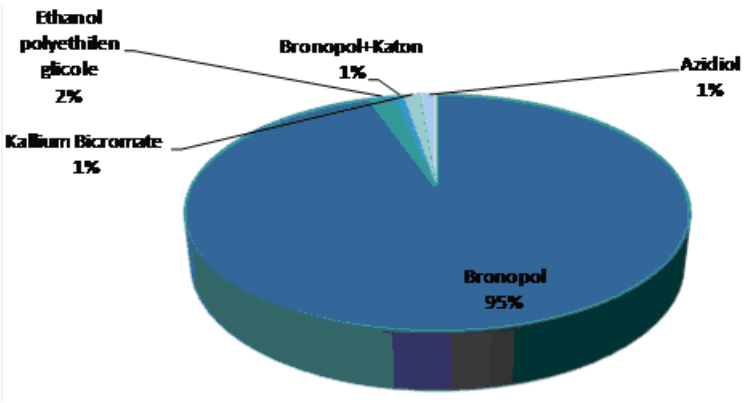

Figure 2. Type of preservative used.

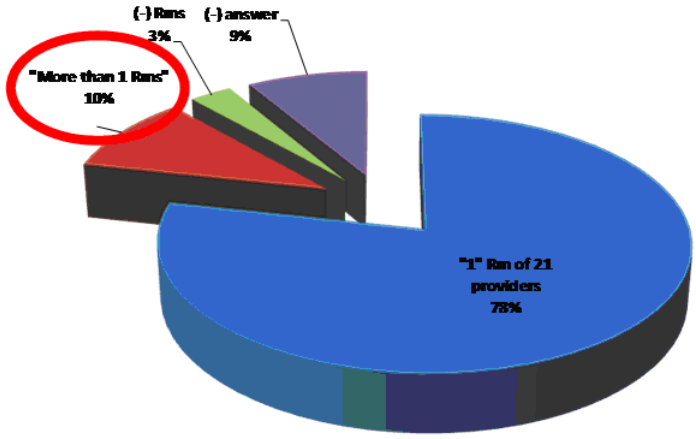

Figure 3. Distribution of the reference materials provided or internal production.

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

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

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

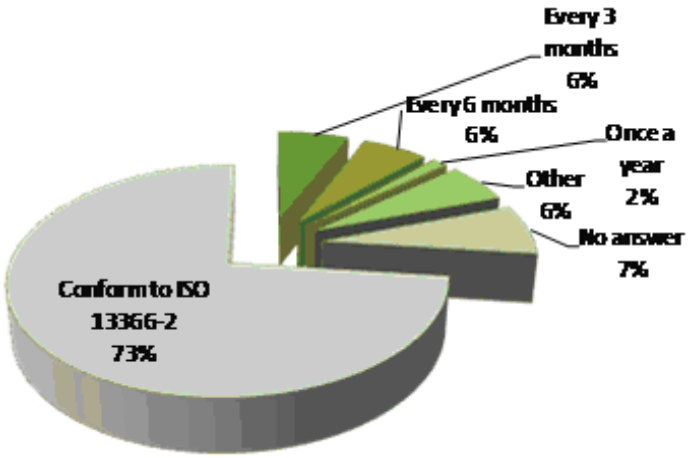

Figure 4. Calibration frequency.

The range of the calibration sets provided by the different reference material providers were divided in 7 categories and 44% of laboratories check the range between 100.000-1.000.000 cells/ml and 44% between 100.000-1.600.000 cells/ml. Some laboratories declared that even if they use a large calibration set (e.g. 100.000-1.800.000 cells/ml) they optimize the accuracy around 400.000 cells/ml

The question 7 "What are the tolerances for eventual adjustment?". Only 30 % of laboratories answer to this question (64 laboratories). Of these 64 laboratories 86 % of laboratories adjust the instrument or call the technical assistence if they are not in the range for slope between 0.95-1.05.

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

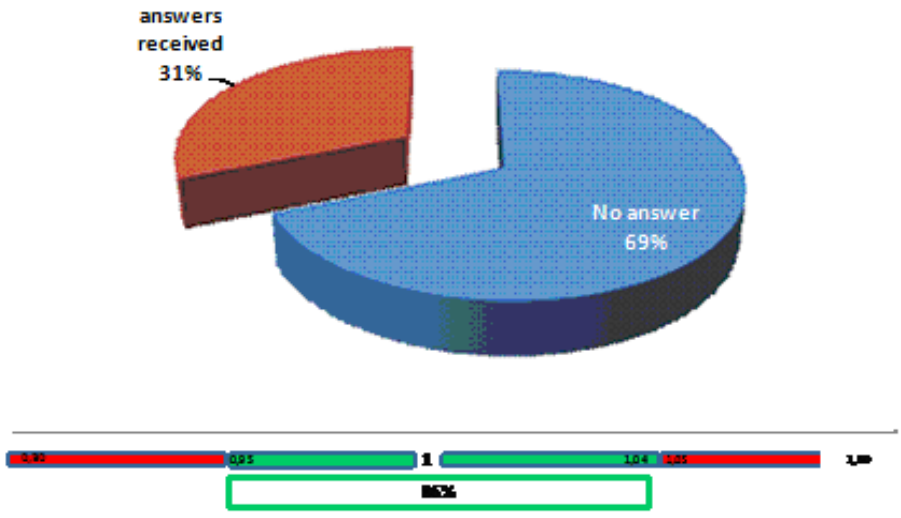

Figure 5. Percentage of laboratories answering on slope adjustment.

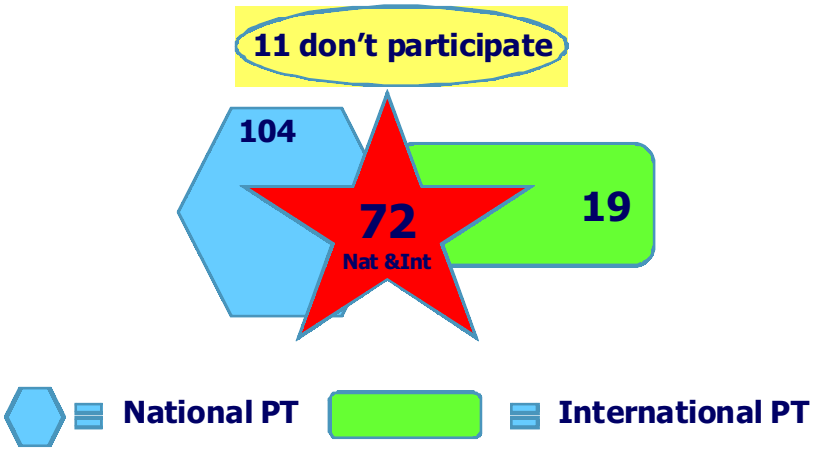

Figure 6. Number of laboratories participating in PT schemes.

Interlaboratory comparison and interlinkages

Question 8 was on "Interlaboratory comparison" on the National or International level. This question was very important to mapping out the distribution and interconnections between the different groups of laboratories. From the previous questions we have seen that some laboratories don't use reference material but prefer to participate in proficiency testing schemes to verify the performance of own instrument/s. 206 laboratories answered according Figure 7 where we can see 104 laboratories participate in national interlaboratory comparison, 19 in international and 72 in both schemes. 11 laboratories don't participate to any comparison.

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

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

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

From the answer received is clear that in the real analytical situation the laboratories participate in national and international proficiency tests with different ranges of concentration Figure 8 and Figure 9.

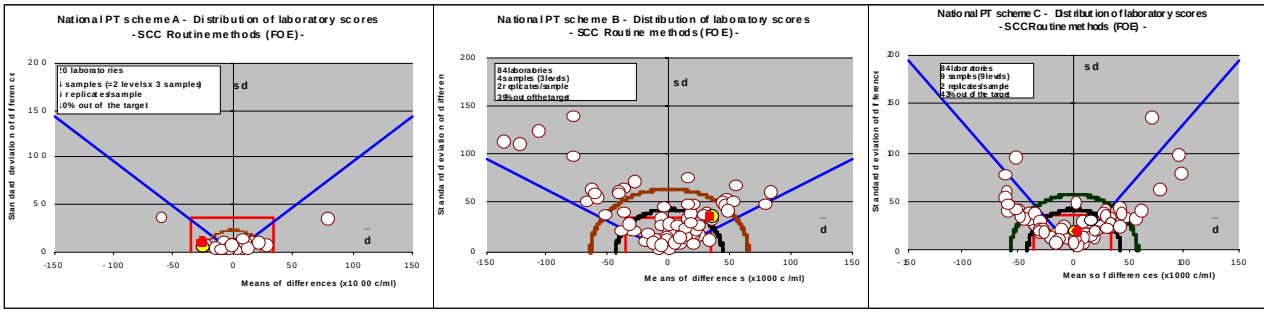

Figure 7. Position of a laboratory that participates simultaneously in different PT schemes.

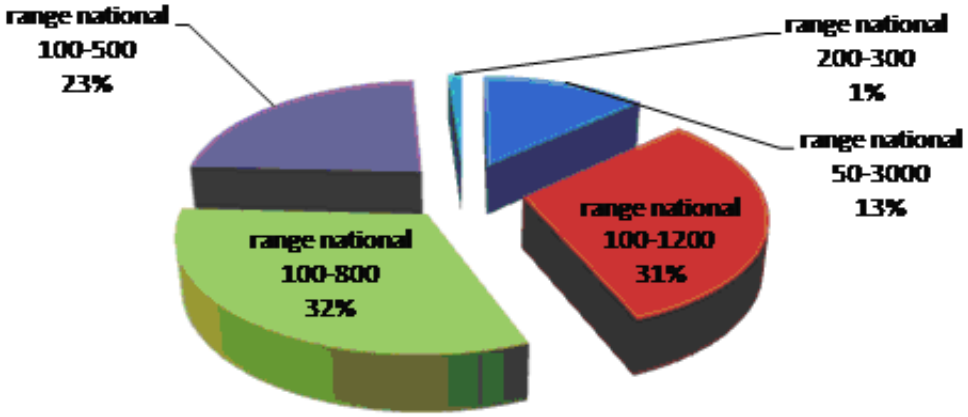

Figure 8. Range of the national PT schemes.

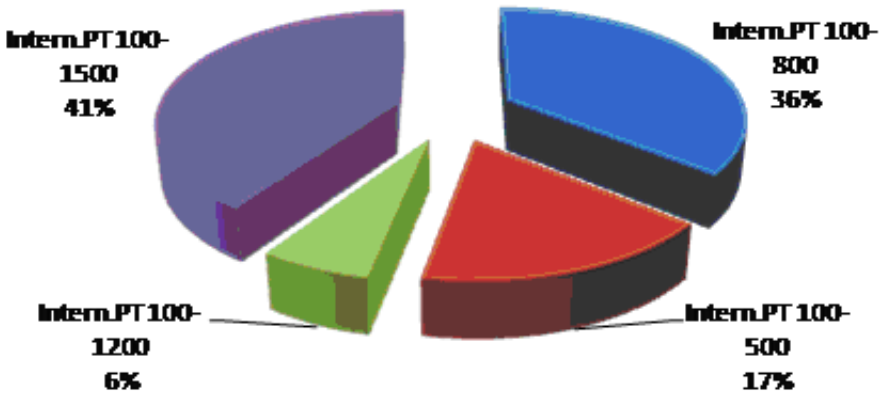

Figure 9. Range of the international PT schemes.

83 % of laboratories participate in scheme with a range from 100.000 to 800.000 cells/ml.

It was very much interesting to draw the map of the different national or international connections of the different countries. Each country has one or more PT schemes and we have focused the attention on the main connections of each continent with the other:

- America: There are interconnections between of Argentina with Bolivia and Brazil, a connection between Canada and USA (Figure 10). America is connected with Europe through Argentina and to Oceania through USA.

  The project could provide the possibility to study the data of Argentina, USA and New Zealand and to create a linkage between the three continents (Figure 11).
- Asia: We have received the answer from Israel and Japan and both of them are interconnected with two different countries in Europe. They participate in two different European PT schemes. Israel takes part also in the ICAR proficiency test. The potentiality to compare these two sets of results could give the possibility to interlink Japan and Israel (Figure 12).
- Oceania: New Zealand was connected with Europe and recently (communicated during the ICAR session in Cork) is connected with USA. The comparison of these two connections initially with Europe and now with USA could be an important information for the project group (Figure 13).
- Africa: Two laboratories are connected with Europe:
- Europe: From Europe we have obtained a very complete picture thanks the 153 received answers from 26 countries. Apparently the map appears complicated because many countries are connected each other trough national and international proficiency tests and several reference materials. We have counted 8 international proficiency schemes and 11 countries are connected with ICAR PT. The further studies of these connections and on the data obtained in the different schemes will be the great adding value of this project.

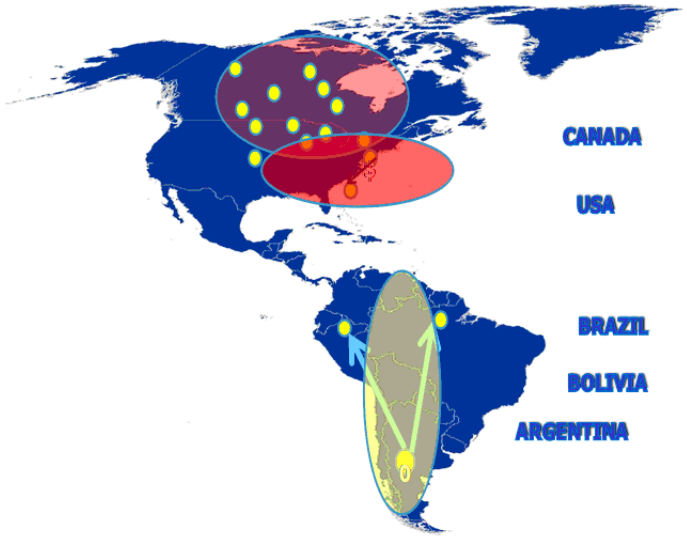

Figure 10. Connections between the countries in America.

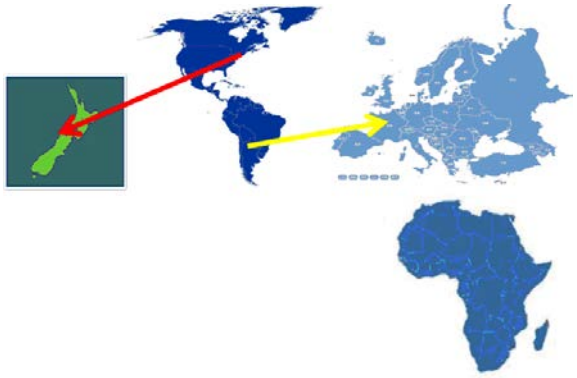

Figure 11. Connections between America and other continents.

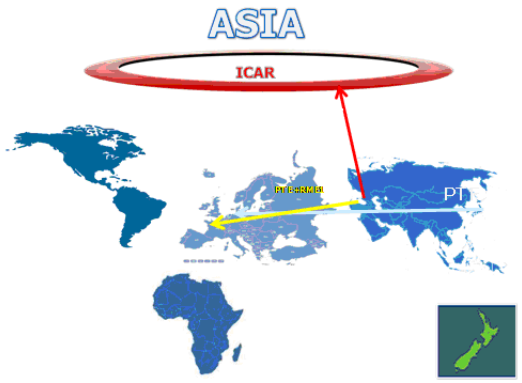

Figure 12. Connections between Asia and other continents.

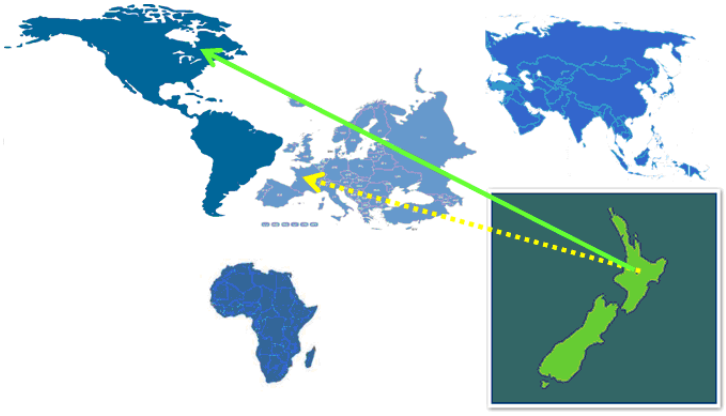

Figure 13. Connections between Oceania and other continents.

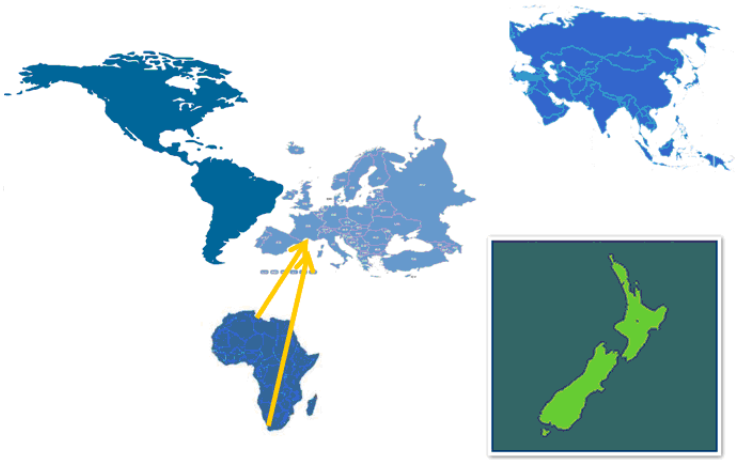

Figure 14. Connections between Africa and other continents.

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

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

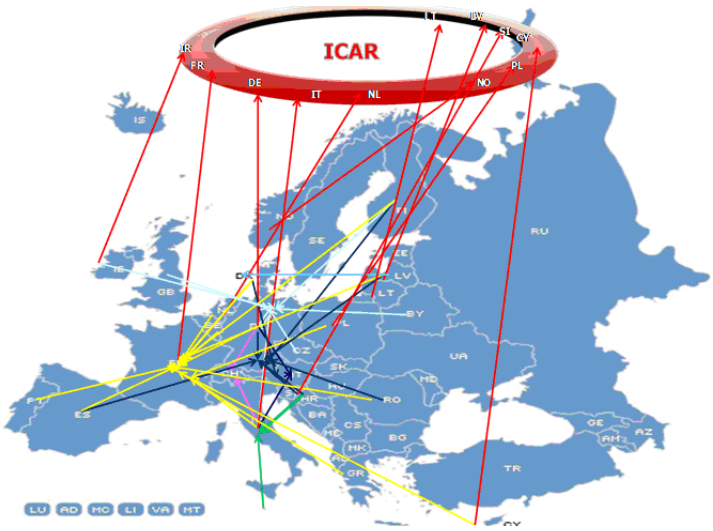

Figure 15. Connections between Europe and other continents.

Conclusion

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

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

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

List of References

Baumgartner. C. 2008. Architecture of reference systems, status quo of somatic cell counting and concept for the implementation of a reference system for somatic cell counting. Bull. IDF 427, p. 15.

Silvia Orlandini, Harrie van den Bijgaart, 2011. Reference system for somatic cell counting in milk. Accred Qual Assur 16:415-420 (2011), DOI 10.1007/s00769-011-0797-7.

ISO 13366-1 | IDF 148-1:2008. Milk - Enumeration of somatic cells, Part 1 - Microscope method (Reference method).

ISO 13366-2 | IDF 148-2:2006. Milk - Enumeration of somatic cells, Part 2 - Guidance on the operation of fluoro-opto-electronic counters.

Newsletter 2 of the IDF/ICAR Project Group on Reference system for somatic cell counting in milk. November 2010.

Newsletter 3 of the IDF/ICAR Project Group on Reference system for somatic cell counting in milk. July 2011.*www.idfdairylaboratories.org/Public/dairylabs/BodyPage.php?ID=3800.*

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

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

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