THE ACTIVITY OF INTERNATIONAL STANDARDISATION OF CECALAIT

At the beginning of 2000, CECALAIT redeployed its experts in standardisation activities to help the dairy industries to respond to the immense changes generated by the globalisation of the trade industry. Important changes in the field of dairy analysis were seen in the nineties. The current objectives are to produce standard methods of analysis with accuracy characteristics established under standardised conditions. This, for the needs of analytical quality assurance and to provide normative information for the Codex Alimentarius within the framework of international trade.

Since 1990, CECALAIT, on top of its services, has a mission of standardisation of analysis methods for milk and dairy products. The activity concerns statistical methods of physico-chemical analysis of milk, statistical tools for calibration and control, and quality assurance concerning milk payment and milk control. In microbiology, only automatic instruments for enumeration of micro-organisms are covered.

EVOLUTION OF STANDARDISATION NEEDS

Since the nineties, quality assurance is increasingly important. Therefore, harmonisation of laboratories by means of proficiency tests and standard reference materials is in process. A mutual recognition of national systems and proficiency tests between countries is being brought about thanks to the first international network of dairy industry reference laboratories.

With standard methods, laboratories will be able to supply their results with uncertainty values. The standards will therefore have to systematically provide accuracy values (repetability and reproductibility), established under perfectly controlled conditions and according to standardised protocols.
Analytical characteristics, concerning quantitative physico-chemical methods have, referentials. However, qualitative or binary methods, quantitative microbiological methods and protocols for evaluation of accuracy are still to be established and standardised. Numerous updates will therefore be necessary upon revision of microbiological and physico-chemical standards.

At the analytical level, important progress has been made in the field of calibration and control of automated milk analysis instruments. In the same way, new methods are appearing – fluoro-opto-electronic methods by flow cytometry, FTIR spectrometry, differential pH-metry, etc…- which will have to be taken into account by standardisation.

Molecular biological methods (such as PCR) developped in order to answer the problems of food safety, are at the origin of the development of many applications in the field of detection and characterisation of pathogenic micro-organisms.

In the antibiotics field, with the increasing diversity of molecules used, consumer health regulations fix maximum limits of residues in dairy products. This has induced a multiplication of rapid detection methods and the development of a specific referential evaluation system by the IDF. In the same way, ISO had to develop international standards for the validation of rapid alternative methods, for pathogenic micro-organisms, due to their fast progression. In parallel, Codex promotes a "criterion approach" to mitigate the deficiency of reference methods in certain fields. So, it is not necessary to have an ISO standardised method thanks the characterisation of internal procedures with defined criteria (linearity, specificity, fidelity, etc…).

REORGANISATION OF THE IDF AND REDEPLOYMENT OF CECALAIT'S EXPERTS

In 2002, the IDF reorganised its activity of analysis of standardisation methods. CECALAIT received a mandate from the dairy interprofession (CNIEL) to ensure an active participation in IDF and AFNOR standardisation. So, 3 engineers invest themselves, for a part of their time, in national and international standardisation activities.

INTERNATIONAL STANDARDISATION LANDSCAPE

The International Dairy Federation (IDF) produces definitions, recommendations and methods which act as specific standards for the dairy industry. In particular, methods of analysis adapted to the specificities of dairy products. These standards have been used by member state dairy organisations.

In parallel, the International Standards Organisation (ISO), produces standard methods of analysis in the majority of the economic branches of industry. The ISO standardisation of analysis methods in milk and dairy products is ensured by the following technical committees : TC 35/SC 5 for physico-chemical methods and TC 34/SC 9 for microbiological methods.

Common rules are necessary in international trade. The Codex Alimentarius, which unites WTO government organisations, ensures this role. The analysis methods and sampling Codex Commission

(AMSCC) and the Milk and dairy products Codex Commission subdivision are dedicated to analysis methods. Experts from different member countries constitute these commissions and the IDF is represented in each one. The Codex does not create standards but uses IDF or ISO standards for the dairy industry.

The European Union had to define a normative framework to apply to the community system and created the European Committee for Standardisation (CEN). For the dairy sector, this committee uses IDF and ISO standards which correspond to its needs but also do standardisation work (Technical committee CEN/TC 302).

In the nineties, a policy of harmonisation and cooperation between the organisations was set up. ISO and IDF experts cooperate. A joint agreement between these two organisations to publish international standards was signed in 1998. Consequently, since 2000, this new form of cooperation has already resulted in the joint publication of a significant number of standards.

FRENCH NATIONAL LEVEL

ALF-FIL France (French IDF members) and AFNOR (French ISO members) work together on draft standards and various questionnaires emitted by IDF and ISO. Several experts, including those from CECALAIT, take part in the work of the two structures. Joint ALF/AFNOR meetings take place to prepare and assess the IDF analytical week. This cooperation works on physico-chemical and microbiological analysis methods.

IDF STANDARDISATION OF ANALYSIS METHODS

The work is carried out by experts of the member states which are designated by their national committees according to their needs. The experts meet at least once a year at the analytical week (IDF/ISO) organised in one of the member states.

For analysis methods, IDF created 5 Standing Committees, or SC, subdivided into work groups called Joint Action Teams or JAT, in which the normative activity is carried out:

1- Main Components in Milk (SC MCM): composed of fat content, nitrogen compounds, lactose and lactates, and moisture groups.

2- Analytical Methods for Additives and Contaminants (SC AMAC): composed of organic contaminants, additives and vitamins, and veterinary residues groups

3- Minor Components and Characterization of Physical Properties (SC MCCPP): composed of minor components, characterisation of heat treatment, physical and rheological properties, and enzymes used in cheese-making groups

4- Microbiological methods of analysis (SC MMA): composed of lactic acid bacteria and starters, and microbiological harmonisation groups

5- Quality Assurance, Statistics of Analytical Data and Sampling (SC QASADS): composed of statistics of analytical data, automated methods, routine methods in quantitative microbiology and sampling groups.

The IDF analysis methods management group conducts the standardisation. This group is constituted of the Chairmen of Standing Committees, the ISO dairy analysis representative and a member of IDF secretariat staff in charge of relationships between experts and National Committees.

IMPLICATION OF CECALAIT IN IDF STANDARDISATION OF ANALYSIS METHODS

CECALAIT is implicated in the activities of 3 IDF Standing Committees: SC MCM, SC MMA and SC QASADS. 3 engineers from CECALAIT participate: P. Rollier, Ph. Trossat and O. Leray. They are experts (contribution and opinion about draft methods) as well as being in charge of drafts (development of standards). They can also be chairman or assistant chairman of work groups:

Standing Committee of main components in milk (SC MCM):

- Fat content group:
The Chairman of this work group is Fred Van Luin. Ph. Trossat is a member. CECALAIT particularly contributes to 2 subjects:

a- Lipolysis in milk (BDI):
In the nineties, the BDI method (according to André Van Reusel's procedure – CRA Gembloux – Belgium), described in Bulletin FIL n° 265, was adopted as the reference method. This procedure was not a standard, it was therefore necessary to work on the standardisation of this method. The responsability of this work remains with Ph. Trossat. A standard should be published in 2005 or 2006.

b- Reference methods for fat content in ewe's and goat's milk:
With the internationalisation of trade, it was essential to start a project to adapt methods that are specific to cow's milk to the specificities of ewe's and goat's

milk. This project is conducted by Cyprus and Italy and CECALAIT follows it attentively. It is in the experimental stage. More complementary tests must be carried out before starting the standardisation process.
The other subjects treated in 2004 by the work group were:

c- Reference method for the determination of sterol content in milkfat and milkfat products,
d- Method for the determination of sterol content in milkfat by GLC,
e- Measurement of peroxide index (revision),
f- Method for the determination of fat content in cheese and processed cheese (revision published in 2004),
g- Reference method for the enumeration of foreign fat by analysis of triglycerides using gas chromatography.

A new subject is under investigation following the proposition of standardisation of a method for the determination of polyunsaturated fatty acids in dairy products (ω-3 et ω-6) by Italy.

- Nitrogen compounds group:
O. Leray (Chairman) and Ph. Trossat are in charge of this group. Two subjects interest and particularly implicate CECALAIT:

a- Reference methods for the determination of nitrogen in ewe's and goat's milk:

As with the determination of fat content, a project to adapt the Kjeldhal method to the specificities of ewe's and goat's milk was set up. The work is conducted by Cyprus and Italy. O. Leray follows this project which is at the end of the preliminary stage.

b- Reference methods for the determination of urea in milk:
There is no reference method for urea content. Preliminary work in 1996 led the IDF to determine a new method : differential pHmetry. Ph. Trossat ensured the finalisation and the publication of this standard in 2004.

The other subjects treated in 2004 by this work group were:
c- Method for the determination of pea and soybean proteins in milk powder,
d- Method for the determination of casein-nitrogen content in milk, direct and indirect (published in 2004),
e- Determination of nitrogen content in milk by rapid semi-micro routine method using the block digestion method (published in 2004),
f- Measurement of seric proteins on caseins by UV spectroscopy,
g- Determination of nitrogen content in cheese by the Kjeldahl method (revision),
h- Determination of casein-nitrogen content and caseinates by the Kjeldahl method (revision).

Due to a multiplication of standards concerning the determination of nitrogen by the Kjeldahl method, the standard ISO 8968/IDF 20 (part 1) is under revision in order to widen its application field to other dairy products. It will gather nitrogen standards which already exist (ex: cheese, caseins, caseinates, etc) and integrate new products such as goat's and ewe's milk. J. Romero (USA) and Ph. Trossat are in charge of the preliminary work. They must define the general scheme and produce a questionnaire on dairy products to be taken into consideration.

- Lactose and lactate group:
This work group is presided by Rachid Kouaouci (CAN) and is kept track of by Ph. Trossat, especially for the following subject:

a- Method for the determination of lactose in milk by differential pHmetry:
There is no reference method for the determination of lactose in raw milk and the HPLC method is not yet standardised. Differential pHmetry presents many advantages (cost, simplicity and automation), that is why the idea to apply it to the determination of lactose was put forward during the IDF analytical week in Lisbon in 1997. The project was validated in 1999. The set up was carried out by the constructor Eurochem (IT) and CRA of Gembloux, with the support of CECALAIT who supplied samples and interlaboratory tests. Finished in 2002, the preliminary stage led to a draft standard which is now in the process of standardisation.

The other subjects treated in 2004 by the work group were:
b- Reference methods for the determination of lactose content in milk and dairy products by HPLC,
c- Determination of citric acid content in cheese and processed cheese by enzymatic method,
d- Determination of lactic acid content and lactates in dried milk by enzymatic method,

- Water group:
This group is presided by G.J. Beutick (NL) for which Ph. Trossat is expert. The subjects treated are:

a- Determination of moisture in fat content of milk by Karl-Fischer method without chloroform,
b- Determination of moisture in dried milk by sweating (published in 2004),
c- Reference method for the determination of dry matter content in cheese and processed cheese,
d- Reference method for the determination of moisture in caseins and caseinates,
e- Determination of dry matter content in yogurt,

f- Routine method for the determination of moisture, non-fat solids and fat content in butter (published in 2004),
g- Determination of freezing point in ewe's and goat's milk.

Two other subjects are studied :
- Determination of moisture content in dairy powders by Karl-Fischer method,
- Determination of titrable acidity in yogurt by potentiometric method.

Standing Committee Quality Assurance, Statistics of Analytical Data and Sampling (SC QASADS):

The **Quality Assurance group** presided by Juan Romero (USA) follows a draft concerning the setting up of a network of reference laboratories to answer the analytical needs of IDF member states. O. Leray conducts this network by the organization of CECALAIT's interlaboratory tests.

- Statistics of analytical data group:
This work group is presided by Hermann Glaeser (UE). Two experts from CECALAIT, O. Leray and Ph. Trossat, take part in this group.
The following subject is under the responsability of CECALAIT:

a- Revision of IDF standard 135B:1991 – Precision characteristics of analytical methods –Collaborative study guidelines:
In the eighties, this standard was produced as a transcription of ISO 5725. Following revisions, significant differences appeared between the ISO referential and the IDF protocol. A harmonisation of these standards is therefore necessary. The results must be the same. The need to keep the IDF standard n° 135 as a practical guide for the dairy industry was expressed. O. Leray has been charged with rendering the two texts compatible. Work is currently in process.

The other subjects treated in 2004 by the work group were:
b- Quality control for colony counting in a microbiological laboratory (parts 1 and 2),
c- Protocol for the definition of the accuracy of microbiological analysis methods,
d- Characterisation of the accuracy of determination using results of several methods,
e- Advice and support to other work groups for interlaboratory tests,
f- Sampling scheme (CCMAS),
g- Guide for the use of reference materials,
h- Use of routine methods in official controls (publication in Bulletin FIL in 2004).

- Automatized method group:
This work group, presided by Harry Van den Bijgaart (NL), is followed by 2 experts from CECALAIT, O. Leray and Ph. Trossat. Two projects are more particularly observed by CECALAIT:

a- Revision of the ISO standard 13366/ IDF 148 – Enumeration of somatic cells in milk:
This standard includes 3 parts: microscopic (reference), Coulter counter and fluoro-opto-electronic method. New techniques have appeared, diversifying the methods used, necessitating the various routine methods to be taken into account more precisely. Furthermore, the application of methods for the counting of somatic cells initially used for cow's milk, to ewe's and goat's milk involves updating the standard with an extension of the application field. So, part 2 will be cancelled; part 1 will be developped particularly concerning the reconnaissance of somatic cells and part 3 will take into account the various configurations of existing methods. This work is organised by Sylvia Orlandini (IT) for part 1 and H. Van den Bijgaart for the other part. O. Leray and Ph. Trossat are members of this work group. Drafts are in the standardisation process.

b- Revision of ISO standard 8196 / IDF 128 – Definition and evaluation of the overall accuracy of indirect methods of milk analysis – Application to calibration procedure and quality control in dairy laboratories:
The development of indirect or instrumental methods for analysis of other components or characteristics of milk and the more frequent use of rapid methods by control services implicate modifications and the extension of the application field of this standard.
An evaluation protocol was proposed to bring about international recognition of new tested alternative methods. This protocol takes the essential of the International Committee of Animal recording (ICAR) evaluation procedure for milk analysers. O. Leray must revise parts 1 and 2 of the standard and write part 3 concerning the evaluation protocol of alternative methods in milk. The standardisation process is in progress.

The other subjects treated in 2004 by the work group were:
c- General information about the determination of urea, caseins, freezing point, etc… by automated indirect methods,
d- Application of near infrared (NIR) for the determination of dry matter and fat content in cheese.

Moreover, the standard ISO 9622/IDF 141 will be revised. Its application field will be extended to Fourier transform mid infrared spectroscopy, to other liquid dairy products and to other milk compounds.

- Routine methods in quantitative microbiology group:
The Chairman of this group is H. Van den Bijgaart (NL). Three experts from CECALAIT participate: O. Leray, P. Rollier and Ph. Trossat. Two subjects particularly interest CECALAIT:

a- Quantitative determination of bacteriological quality – Conversion :
It was necessary to group the instrumental methods of milk payment and the reference method IDF 100. The term "conversion method" is used in order to express the translation of instrumental signals (pulses) to Colony Forming Units (CFU). The principle and the method, which is equivalent to a calibration procedure for physico-chemical indirect methods, are described in this document. It was published in 2004.

b- Quantitative determination of bacteriological quality – Guidelines for the evaluation of routine methods:
IDF standard 161 and ISO 16140 must be equivalent in order to draw the same conclusions for automatic validated apparatus. So, Gertraud Suhren (DE) with Ph. Trossat and P. Rollier are working on a parallel of these 2 standards.

Standing Committee microbiological analysis methods (SC MMA):

- Harmonisation in microbiology group:
The work group produces and revises microbiological standards. It introduces accuracy values and ensures harmonisation with the existing IDF horizontal standards. If IDF and ISO standards are compatible, IDF standards are cancelled. The chairman is H. Becker (DE) and P. Rollier ensures the participation for CECALAIT. She is more particularly in charge of 3 projects:

a- Method for the enumeration of coagulase positive staphylococci by colony counting at 37°C in milk and milk products:
The 6888-1 and 2 standards have been amended to take into account specificities of IDF 145 / ISO 11867 standard which will be deleted.

b- Analysis of performance of existing methods concerning the enumeration *of Pseudomonas spp.* to elaborate a new method adapted to dairy products:
The objective is to revise the draft horizontal standard ISO 13720. P. Rollier manages this project. Complementary experimentations are necessary at the IDF and ISO level. The elaboration of the draft is on standby.

c- General guide for sample preparation, initial suspensions and decimal dilutions for microbiological analyses in milk and dairy products:
A new revision of IDF 122/ISO 8261 is underway because of the incompatibility between many methods described in the standard and laboratory practices. P. Rollier is in charge of this new project which began in 2005.

The other subjects treated in 2004 by the work group were:
d- Enumeration of presumed *Escherichia coli* by MPN technique (part 1), by MPN technique with MUG (part 2), by colony counting on a membrane (part 3) in milk and dairy products,
e- Enumeration of coliforms by colony counting at 30°C (part 1), by MPN technique (part 2),
f- Enumeration of *Bacillus cereus* by MPN technique in dried dairy products,
g- Detection of *Listeria monocytogenes* in milk and milk products,
h- General rules for the microbiological examination of food and feedstuffs,
i- Enumeration of micro-organisms by the calibrated inoculation loop technique at 30°C,
j- Enumeration of psychrotrophic micro-organisms by rapid colony count technique 25 h at 21°C in milk,
k- Detection of thermonuclease produced by coagulase positive staphylococci in milk and dairy products.

Many other subjects are under consideration:
- Enumeration of *Listeria monocytogenes* by colony counting in milk and dairy products,
- Detection of coagulase positive staphylococci by MPN technique in milk and dairy products,
- Horizontal method for the detection and the enumeration of *Campylobacter* at 41,5°C – Part 1 : Detection method.
Proposition of new subjects:
- Detection of *Enterobacter sakazakii* in dried infant products

The other Standing Committees:

Many other experts from AFSSA, INRA or various private organisations participate in the other Standing Committees.

CONCLUSION

CECALAIT participates actively with the national and international standardisation bodies in order to point out the positions of the French dairy industry.