

## INTERNATIONAL HARMONIZATION OF MICROBIOLOGICAL METHODS

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It is my great honor and pleasure to be here today and I would like to thank very warmly the organizers, Josep and Marta, for inviting me to present the introductory paper of the IX Workshop on Rapid Methods and Automation held in Barcelona .

I must say that the world of rapid methods in which I have been submerged a very long time ago is really a fascinating one ,in terms of techniques but also of scientists : they are all so enthusiastic and eager to find new ways of working in Food microbiology in order to decrease the cost of analysis while increasing the number of samples! However, it took a long time for those alternative methods to be officially accepted ; one of the reasons , of course, is that the results have to be recognized by the scientific community as well by official bodies ; considering the fact that hamonization of traditional methods was and remains a difficult topic , it is very easy to understand that, when rapid methods did appear on the market, other problems of acceptance had to be faced : that was unavoidable

In spite of their importance, especially for international trade, different aspects of harmonization of microbiological methods are not widely known .and, even if my knowledge of the topic is far to be perfect , I thought it would be useful , as an introductory presentation to that IX workshop held in Barcelona , to share with you a few data and comments on that topic and I propose to divide my presentation into four parts:

- In a first time , I would like to present some general considerations and definitions
- In a second time, if you allow me to do so, I will tell you a few words concerning the reasons which led me to be involved in the field of Harmonization of Microbiological Methods.
- Then, I will describe some of the main Standardization bodies around the world.

Finally and before my conclusion, I would like to focus on recent approaches which show the willingness of international harmonization in Food microbiology .

### I- General considerations and definitions

Harmonization , in a first approach, relates to music, to equilibrium between different registers but it concerns different fields and we can find examples of harmonization everywhere .

According to ISO ( the International Standards Organization about which I will tell a few words very soon ), standards are “ documented agreements containing technical specifications or other precise criteria to be used consistently as rules, guidelines or definitions of characteristics to ensure that materials, products, processes and services are fit for their purpose” ;

Concerning Food microbiology harmonisation, we all know the necessary conditions to be filled for a comparable evaluation of the food quality .

- the food samples examined have to be representative of the food examined , in fact, I should say “should be”, in terms of size of the samples ,as it is really impossible , for practical reasons , to analyse a sample of a sufficient size ( with the help of statisticians we can only try to be close to the reality ) . However , we can always follow the same rules in terms of type of sample and also of methods of analysis , which is very important .

Considering the type of sample , I will explain from a simple example: if we want to define the contamination of a poultry carcass , examining the skin or the muscle is not the same thing and results will surely be very different .

Considering the methods of analysis, harmonization is of upper importance to obtain the same or, at least, equivalent results . In Europe , a special emphasis on those problems has been given and can be seen when reading the paper on microbiological criteria for food published 15 December 2005 in the Official Journal of

European Communities ; however, everybody knows that the harmonization of methods remains a difficult task and we can remember that, a few decades ago , there were a lot of techniques used to detect or enumerate the same type of microorganism and each microbiologist wanted to keep his ( her) own method....

## II- Entering in the field of Harmonization of Microbiological Methods

A long time ago, when I began to work , I was in Ploufragan ( Brittany ) where I have been working for 27 years .At that time , there was no laboratory in the place, only a control lab , far from a few kilometers . My project was to improve the quality of poultry products, in order to help industrials to improve their techniques and increase the shelflife of poultry meat ( which was sold as refrigerated while the conditions of processing were not so good) and to improve the hygienic quality of their products . ...

Different options had to be taken concerning

- The types and size of the samples
- The types of microorganisms to be studied and the methods of analysis

Many years later, I can't do otherwise than to observe that, without any necessity or willingness of standardization; from the beginnings, we had probably felt its necessity ..

We were also convinced of the necessity of studying a high number of samples in order to avoid "precision in error" ; in fact , we had no idea of the poultry contamination and we had in mind the objective to set up general laws which regulate the level of contaminations .. determine critical points and try to improve the microbiological quality . Later on, we decided to try determining the origin of contaminations ; then to look at pathogenic microorganisms along the production lines ( "from farm to fork")

All that work was very expensive and the necessity of using alternative methods became obvious from that period . In France, some scientists had similar preoccupations and we began to exchange with Prs C.Bourgeois and P.Mafart in Quimper with Pr H.Leclerc in Lille . but the most decisive for our future work was my participation in a symposium which was held in Kiel(D) in 1974 . I can't forget it.. I did not present anything on that day ; I was just learning and listening at the different papers when , suddenly , we had a very interesting and unusual presentation : the speaker , a young American Chinese was quite "dancing" , showing beautiful slides : the miniaturized methods used to detect or enumerate microorganisms from poultry was the subject of his presentation : he was using microplates instead of tubes and a micro-inoculator instead of platina loops ...He had a very simple principle" THINK SMALL" . As I was working on poultry meat microbiology and was very much interested by rapid and economic methods in order to study a large number of strains simultaneously , I met Dr Fung at the end of the session , asking for informations and reprints ; I must say it was the beginning of a long story .. first because he sent me reprints and even his thesis and, from that time we became very good friends but I must say, too , that it opened me new horizons, new contacts and a willingness to communicate about the possibilities which were offered to food microbiologists : they could use rapid method which were , till that period, used in the medical field only ... for me , it was the beginning of a long story in science and friendship .....Dr Fung visited our Institute in 1976 and , from that time , miniaturized methods were introduced in our laboratory and used to identify a lot of strains to trace Salmonella , then different types of pathogenic microorganisms " from farm to fork"; we were so enthusiastic with those methods that we presented the technique as well as the results during different meetings in different laboratories : we wanted those methods be adopted by the scientific community ... and were somewhat successful .

Here, I would like to tell you one short story :

I remember the visit of a politician in our lab : I was explaining him that with a low quantity of media, in a very short time, we could examine far more strains than usual . He told me : in that case , you will decrease your staff! And I answered : NO ! the same technicians will study far more than usual , that'all ! he said : OK...

As you surely know, from the idea of Dr Fung ( THINK SMALL ! ), a lot of kits appeared on the market ; you are aware of the present situation ...but the following is also a very beautiful story , i.e the creation of courses showing rapid methods in microbiology all around the world . I want to remember : once , I was in K.State University and , after my presentation, I met a young Spanish scientist , eager of knowledge and new informations ; that was just when Food safety Agencies in Europe were being organized ; he told me : I was very interested in your talk but it would have been interesting to say more .. he looked somewhat disappointed .. Was it the reason why he invited me different times in Barcelona to present the introductive paper to the Spanish workshop ? I don't know but, anyway, I am very happy to be here today and I think that what we are living is a perfect example of a chain in Science ..

However, the necessity of **validating** those methods in order to make them accepted became obvious very early .and, for me, personally , it was a big concern . In Afnor , the question of rapid, alternative methods became one branch of standardization in the 1980's .

As I was concerned by harmonization, not only for traditional methods but also, and mainly for rapid methods , I was introduced, step by step, in that world of standardization : at the time I was in charge of the Central Food Hygiene Laboratory in Paris, I was requested to be the convenor of working group 6 of the technical committee 275 of CEN ( Comité Européen de Normalisation) ; at that time, I also participated in some meetings of Codex alimentarius ( to mention those related to standardization) In 1995, that group of CEN became concerned by rapid methods . Personally, I retired from CEN in 2005 but I remain in contact and , in 2009, I had the great pleasure to join my previous colleagues for the joined meeting ISO/CEN in Valencia ( Spain)

### III- Short history and description of some international organizations connected with Food microbiology

The **first historic writings** show that governments took interest in codifications of rules set up to protect the consumer , but the first laws , concerning mainly the chemical purity appeared during the first part of the 19<sup>th</sup> century . AOAC ( **created as the Association of Agricultural Official Chemists** ) was created in 1884 but is more a validation than a standardization body ; then , at the beginning of the 20<sup>th</sup> century , one can mention the existence of **IDF ( International Dairy Federation )** , of **BSI ( British Standards Institute )** , etc... but the fruitful period appeared just after the second World War and I shall focus on them a few minutes .

#### - **ISO ( International Standards Organization)**

As you probably know , different official bodies are concerned by standardization under different aspects for different types of production and everybody knows ISO, i.e the International Standards Organisation ; this organization was created in October 1946; its seat is located in Geneva ( CH ) ; the creation results from the fusion of two organizations :

ISA which was the International Federation of National Associations of Standardization, founded in New-York in 1926 and

UNSC, i.e Committee for coordination of standardization of United Nations, created in 1944 .

The first national Assembly was held in 1949 in the great amphitheater in Sorbonne ( Paris)

ISO is composed of 247 committees . All standards are obtained by consensus ; however , they are not mandatory ..The technical committee in charge of Food microbiology is TC34 / SC9 ; the actual president is Bertrand Lombard (F); the meetings are held in a different country each year ; for example , in 2009, the meeting was held in Valencia ( Spain) ; In May 2010 ,so quite recently, the meeting was held in Argentina ; as usual, it was a joined ISO/CEN meeting

**CEN, Comité Européen de Normalisation** ( *in English "European Committee for Standardization "* ) has been created in 1961 in order to harmonize the standards elaborated in Europe ; that means that the standards are mandatory in all countries of EC ( at the contrary, the standards which are elaborated by ISO are facultative , which means a great difference ... All members are members of ISO as well . The seat of CEN is located in Brussels ( B ) .In the beginnings , it was created by the national organisms for standardization from France, Germany and Benelux countries .Nowadays, the full members are the 27 countries of EU and the three contries of AELE ( Association Européenne de Libre Echange) which

own such an organism ( Switzerland, Norway and Island ). CEN elaborates technical standards in favor of international trade .

CEN/TC275/WG6 was created in 1993 and I was nominated as the convenor ; nowadays , from July 2005, Alexandre Leclercq from Institut Pasteur in Paris , is in charge of the group .

From the beginnings, one main principle has been followed during the work of this group , i.e the Vienna Agreement ; this agreement requires that, as often as possible, ISO methods are taken into account .

In order to avoid any overlap, there is also an agreement between different groups of CEN that only one group is in charge of a particular method ( the “ Vienna agreement” ) . For example, TC302 in charge of milk and dairy products analysis may choose one specific technique . In this case, it requests TC 275/WG6 to refer to this specific technique in the standard method . The necessity of taking into account the experience of other groups around the world, for example AOAC and IDF ( International Dairy Federation) , has also been emphasized from the beginning and , presently, the basis for a good cooperation has been set up .

In order to maintain a good international cooperation, one part of the meeting concerns the liaison with other organizations , ie : International Dairy Federation ( IDF) Codex Committee on Food Hygiene , AOAC ( Association of Official Analytical Chemists), WHO ( World’Health Organisation ) , IUMS ( International Union of Microbiological Societies ) , OIE, ( Office International des Epizooties ) and also NMKL ( cooperation agreement between NMKL ( Nordic Committee on Food Analysis ) and ISO/TC34SC9)and ISO TC347 and ISO TC147/SC4 Water microbiology .

#### - Codex alimentarius

Of course, all of you have heard of Codex alimentarius : the name means a food code and is a compilation of standards for food , which can be applied in all countries . Codex has been created in 1962 when the organization of United Nations for Food and Agriculture ( FAO ) and the World’s Health organization decided it was necessary to elaborate international standards which may be used by the Food industry which was in full expansion in order to protect consumer’s health . in fact , in the first volume , it is said that the objectives of Codex alimentarius are : “ to guide and promote the elaboration and setting up of definitions and criteria for food, in order to contribute to their harmonization and facilitate international exchanges .

Codex alimentarius has a noticeable effect on quality and safety of food around the world ; it has allowed , everywhere, to improve the standards which are applied at different steps of the food chain and to increase a lot the international exchanges .

In the Codex alimentarius, there is high number of committees , i.e two groups of world committees, one on general problems, the other on food products. There are also some regional committees .

Food hygienists may be experts in many of those groups ; personally , I participated different times in the committee “ Food Hygiene” for which USA are the leader .

All committees work in strong collaboration with scientific organizations in order to elaborate standards and recommendations .

As an example , I would like to remember some sentences of one paper written in the “International Journal of Food Microbiology” in 1998 :

During the 21<sup>st</sup> session of Codex Alimentarius which was held in Rome, Italy in July 1995, the Commission was invited to adapt as Codex general standards, a number of draft texts submitted at step 8 of the Uniform Procedure for the Laboratory Codex Standards and related texts – i.e .general standards for contaminants and toxins in foods – methods of analysis and sampling “. Chemical contaminants were considered first but , *when, under CEN collaborative studies were carried out and that precision parameters were introduced into the ISO standards , they have been transmitted to Codex Alimentarius , so creating an international consensus .*

#### IV – Harmonisation of microbiological methods and of procedures for validation

**The harmonization of methods** is the major task of the standardization bodies.

“*Stricto sensu*”, International standards are exclusively elaborated by ISO ( the technical work of ISO is highly decentralised and carried out in technical committees , subcommittees and working groups as TC34/SC9 for microbiological methods)

Different national bodies : Afnor, BSI, DIN... in Europe, ANSI in USA ( ANSI is not an active member of ISO but close relationships have been established in the Food microbiological sector .

As an example, I'll say a few words about the elaboration of a ISO standard :

It always take into account the following principles :

- The consensus : all points of view are considered, whichever their origin .
- At the industry scale, the solutions must aim to satisfy all concerned parties around the world .
- It depends on the willingness of the partners

Three phases of elaboration can be described :

- The need of a standard is usually submitted by a sector of the industry which informs a national member of that need ; the national member informs ISO . When the need of a standard for a given technique, product ... has been approved and accepted, the following task is to define the technical subject of the future standard . That phase is carried on within working groups composed of experts of countries interested in the question .
- When an agreement concerning the technical aspects is obtained, a second phase begins : it is the consensus phase
- The last part of the work concerns the formal approval of the draft of international standard ( the criteria are that two thirds of the ISO members who have participated in the elaboration procedure must approve the draft ; also 75% of the voting members must approve it ) . Then , the draft is accepted as an International ISO Standard . It is also possible, presently, to publish drafts at different stages .

It seems important to know that some standards, obtained by consensus had not been validated by interlaboratory tests ; but the necessity of their validation appeared obvious especially for the acceptance of alternative methods : as it is always necessary to compare a given method with a reference one . This is the reason why , under WG6 of CEN/TC275 and quite a short time after the creation of the working group , a proposal for a project entitled " Evaluation of microbiological methods for detection and/or enumeration of microbiological contaminants in foods " was presented and then accepted as an EU project ( SMT4/CT96-2098 ) . The trials were carried on from 1996 to 2000 ; the results for all types of contaminants studied have been accepted by the equivalent group of ISO and published in the " International Journal of Food Microbiology " and later on, adapted as Codex standards . They have become Reference methods, which was essential for the development of alternative methods ...

From that time, we had reference methods for *Bacillus cereus* , *Staphylococcus aureus* , *Clostridium perfringens* , *Listeria monocytogenes* enumeration , for *Salmonella* and *Listeria monocytogenes* detection . The results obtained would make possible the comparison of alternative methods with the reference ones and, hopefully, their validation. The results obtained during that SMT Project were published in the International Journal of Food Microbiology .

**At that time also , a close cooperation began between CEN/ISO and AOAC and I'll will always remember one day in The Hague (NL) when we discussed around a table about the possible organization of common experiments concerning European and US laboratories ( that was a big concern as it was a European Project !)**

Finally some trials were organized by European and US laboratories in order to compare the ISO method for the detection of *Salmonella* ( 6579/ 2002)with the AOAC method .

**Finally, ISO 6579:2002 was recommended to be adopted as official First Action for the analysis of fresh cheese, fresh chilled and frozen poultry and dried egg product .I shall go back on that point in a few minutes. For the first time, one could read " Hands touch over the sea "**

Another point has to be added . **How to introduce new technologies** in that world of traditional methods?; that was also the subject of many discussions . Finally, An important resolution was taken during the joined meeting of ISO/TC34/SC9 and CEN/TC275/WG6 held in Parma (It) in April 2004.

*“ Each time a standard method is being revised , the possibility of using new technologies , including PCR, must be examined by comparing results with those obtained when using the official conventional method .*

*For a given microorganism, in order to complete the existing method, the development of standardised methods based on new technologies can be proposed when the purpose to be obtained ( for example pathogenicity level ) makes it necessary .*

*When new technologies , including PCR, are used as alternative methods , they must be validated against the reference method .*

**Those sentences look probably as quite simple ,but I am sure you cannot imagine the number of hours of discussions which were necessary to obtain a consensus : that is “ international cooperation “**

But the hours of discussions were very fruitful ..as , finally, rapid methods were accepted according to the EC regulation 2073 15 December 2005 concerning microbiological criteria :

*“ Test results are dependent on the analytical method used and, therefore, a given reference method should be associated with each microbiological criterion . However, Food business operators have the possibility to use analytical methods other than the reference method, in particular more rapid methods, as long as the use of these alternative methods provide equivalent results “*

#### **So, alternative methods were finally, officially accepted in the EC**

#### **IV- Recent approaches in the harmonization of Food microbiological methods**

But , of course, difficulties were not over .. **How to validate alternative methods** was the first question.

As we have said , a lot of alternative methods can be found on the market and the present workshop is devoted to those methods , but two main systems of validation do exist :

- The AOAC Validation process
- The standard EN/ISO 16140

They both are composed of two phases : A method comparison ( Pre-collaborative study)  
An Interlaboratory Collaborative Study

Of course there are some differences between the two systems concerning especially the number of matrices ( 6 for AOAC , 1 for ISO/16140) ; the reference methods are also different ( USDA, FDA, AOAC for AOAC, ISO for EN/ISO/16140) but the principles are similar, of course .Significant efforts have been realized for a mutual recognition .

I would also like to add a few words about recent advances concerning **MicroVal** .

**-MicroVal** is an “Eurekat” project ( the Eureka programme was set upon 1985 to stimulate cross- border technological cooperation and advancement throughout Europe ) . it started in 1993 with the aim of setting up European validation procedure in four years time and furthermore of creating such conditions that the results of the procedure would be accepted as far as possible by all interested parties in Europe . Standardization and certification play important roles in this respect .

MicroVal started as a Dutch-French initiative . The project was ended by 21 full partners from 7 countries . Let’s me say a few words about the three working groups which were set up :

-Working group 1 studied information on the validation used or proposed by AFNOR ( Association Française de Normalisation) , The AOAC Research Institute, the European Community ( EC) , the International Dairy Federation (IDF) and the International Union of Pure and Applied Chemistry( IUPAC). The final report of the first working group was presented at the First Annual Meeting in Paris, 1994.

- Working group 2 drew up the general rules for the organization of the validation procedure and the Validation Certification Scheme .

-The third group started in parallel with the second and focused on drawing up the technical rules for validation, the test protocol and the organization of the trial validations in the second stage of the project .

- The fourth working group looked after the financial aspects of the project .

The General Rules describe the methods and the organization to be used for the European certification of alternative methods for the food and drink industry by an independent organization : MicroVal certification .

In the spring 1996, the MicroVal steering committee started the procedure for European Standardization by forwarding to the European Standardization Committee (CEN), a proposal for a European Standard based on criteria developed by MicroVal for the validation of alternative microbiological methods . This proposal was accepted in June 1996 by CEN /TC 275/WG6 as a new work item for a European Standard " Protocol for the validation of alternative microbiological methods" based on the two MicroVal documents " General" and " Technical" rules for Validation criteria .. That was the beginning of MicroVal ..

One really important event is the acceptance of the results of **combined validations Microval / AOAC** , last November during the 2<sup>nd</sup> MicroVal Symposium which was held in the Hague (NL) . What does it mean ?

It means that ( cf Michele Smoot ):

AOAC and MicroVal joined forces to combine a MicroVal ( basis EN/ISO/16140) certificate with AOAC Validation ( Official Methods of Analysis and/or Performance Tested Methods)

Both organizations seek to fulfill their own requirements with respect to the procedures , rules, fees, etc, while designing a protocol that fits both AOAC and MicroVal requirements

Those two examples I just mentioned seem to me to be very promising ....

**What to say as a conclusion ?**

Everybody knows that harmonization of Food microbiological methods will surely take a very long time . even if one knows that " a broad acceptance of microbiological methods and associated results would facilitate knowledge exchange and would speed up the implementation of new methods / technologies across appropriate food matrices" ( Michele Smoot, IAAP Europe )

However, I think we can be somewhat optimistic : even if a recent observer could think that the progress is very low, alternative methods seem to be now fully accepted quite everywhere; some good reference methods do exist , even if the work is not complete ..., some approaches to harmonize the systems of validation do exist and even combined validations have been set up !

And all that international Harmonization of Microbiological Methods will take a growing importance with the extension of Food Microbiology to the primary production ..We may hope that , in the future , the situation will improve day by day ...May we be allowed to consider International Harmonization of Microbiological Methods as a wonderful school to find the way of consensus in different situations of human lives ?