

**A successful European event : AFNOR Validation Symposium
Brussels, June 19, 2008**



Bertrand Lombard, the Symposium Convenor, is involved in the AFNOR Validation Scheme representing the AFSSA (French Food Safety Agency) in the AFNOR Validation Commission. He has also participated in the Eukeka MicroVal project from 1994 to 1998 representing AFNOR at that time and having led the group in charge of developing the technical protocol for validation. Currently, as Chairman of ISO/TC 34/SC9, he is involved in the revision of validation protocol (EN ISO 16140 standard), which is conducted within the responsibility of this structure and he is also convenor of WG2 (statistics) of ISO/TC 34/SC9.

Bertrand Lombard emphasized on the fact that comments from AFNOR Validation Scheme were important to ISO/TC 34/SC9 to take the resolution to revise EN ISO 16140. All the alternative methods already validated according to this standard via the AFNOR Validation scheme give a large amount of data which are very useful to the ISO working groups in the revision of the EN ISO 16140 protocol.

Ari HORMAN (DG SANCO, E2) recalled the aim of EU Regulation on microbiology of foodstuffs and underlined the need of rapid methods on the market as they are a real advantage for food operators. He recalled the three main requirements stated by regulation (EC)2073/2005 in order for rapid methods to be recognized at the European level :

1. comparison to ISO/CEN reference method,
2. validation according to the validation protocol stated in standard EN ISO 16140 (or similar),
3. certification by a third party.

Ari HORMAN emphasized on the need to respect a certification scheme : the validation protocol must be used in the context of certification.

Then **Valentine DIGONNET** (AFNOR Certification) gave full details on the AFNOR Validation scheme in Food Microbiology. Certification rules fully comply with the requirements of EU Regulation so that AFNOR validated methods are recognized at the European level. Suppliers are granted with the AFNOR Validation European mark for the corresponding methods. This is a collective mark registered all through Europe.

In the future, AFNOR Certification wishes to continue harmonization with other European or international certification schemes, resolving the main issues currently on discussion :

- Use of same validation protocol (EN ISO 16140)
- Use of same reference methods (ISO and CEN standardized methods of analysis)
- Use of similar matrices and use of natural contamination when possible during the preliminary study (...)

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The two most famous expert laboratories presented the contents and interpretation of validation protocol EN ISO 16140 used in the AFNOR Validation Scheme.

For qualitative studies, **Virginie EWE** (Institut Pasteur Lille) underlined the importance of natural contamination, which is not mandatory in EN ISO 16140. A minimum of naturally contaminated samples are required by AFNOR Validation protocol (25% for *Salmonella* and 50 % for *Listeria*). For users and microbiologists, this is a major advantage of AFNOR Validation Scheme compared to AOAC schemes for example which only uses artificially contaminated samples.

Virginie EWE recalled that there were no acceptance criteria in standard EN ISO 16140, so that the statistical tests proposed are systematically studied by the AFNOR Validation technical board which gives advice on results.

Danièle SOHIER (ADRIA Development) talked about the EN ISO 16140 protocol for the quantitative methods validation study. Several examples clearly showed that the reliability of the proposed statistical tests could be discussed. She suggested to revise some chapters of the standard in order to make it more user friendly, and mentioned that the standard revision is now in progress according to the AFNOR Validation Technical Board recommendations. Users are invited to refer to the summary reports which provide deep information and explanations on the validation study results.

Then **Helmut STEINKAMP** (D.I.L.) showed his experience as a participant laboratory for several AFNOR Validation inter-laboratory studies. As a consequence of the participation of DIL in these studies, about 50% of laboratory results come from AFNOR. As a matter of fact DIL is particularly interested in PCR techniques and collaborates a lot with Bio-Rad in this field.

The two biggest suppliers of AFNOR validated methods showed their interest in a harmonized European validation of kits.

At first **Marie-Pierre COPIN** (3M Health Microbiology Europe) showed to the participants the great number of recognitions granted to 3M by AOAC, AFNOR Validation and NordVal. The Petrifilm range of kits was completed in 2006 with Biotrace company acquisition, by a pathogen tests range : Tecra (ELISA and immunocapture tests), and an hygien test range. Common history between AFNOR Validation and 3M Health started in 1989 with the first certification granted to a Petrifilm test and goes on in 2008 with a large number of AFNOR validated methods.

As one of the major suppliers of kits worldwide; 3M appreciated the market progress in Europe so as to get a unique scheme based on international EN ISO 16140 standard. A more common scheme with AOAC would be ideal.

Regarding the AFNOR Validation Scheme, Marie-Pierre COPIN added that she really appreciated having the possibility to choose the competent expert laboratory and the third party certification body to get their methods validated (system improvement, speed ...)

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Then **Raffaella GIARDINO** (bioMérieux) showed the current list of AFNOR validated methods, coming to 21 methods in 2008 which are divided into 13 qualitative and 8 quantitative methods : 11 VIDAS, 4 TEMPO, 5 culture media and 1 molecular hybridation method.

Giving an example of validations studies results realized on TEMPO, she emphasized on the importance of the practicability criteria which is specific to AFNOR Validation Scheme. It is important for users to know what benefit they can get from the method in terms of user friendliness, time to results or time to operator training for instance.

Raffaella GIARDINO talked about the great experience of the AFNOR Validation technical board among the members of which good sense prevails on purely statistical results.

She also testified on behalf of the general coherence of the Validation Scheme, starting from the submission of data file, taking into account content of the validation certificate and summary report as well as validation management by AFNOR Certification, audits and overall costs.

As an evidence of the users's interest, **John MARUGG** (Nestlé Research Center) talked about the particular need of Nestlé as a food industry. There is a big need for rapid methods although many problems may occur during the analysis of samples such as complex matrices, low numbers of strains in samples, intrinsic bacterial flora which may hinder identification or injured cells and dead cells.

Nestlé stated a list of all requirements they need in order to conduct analysis of food samples, such as the use of validated methods, low costs, easy training or automation,...

Nestlé created NesVal which is a successful coordinated and systematic approach for method evaluation, approval and implementation. NesVal approved methods are mainly based upon AFNOR validated methods according to EN ISO 16140 standards. NesVal also develops proper methods. An easier variant of ISO 6579 was developed recently for which current ISO 16140 validation is going on.

Due to the number of methods used and the large number of samples to analyse in all food categories, Nestlé wishes harmonization of reference methods (ISO/CEN versus AOAC) and also international validation procedures.

Considering AFNOR Validation, John MARUGG suggested that the scheme would include more food matrices that are relevant for Nestlé as well as relevant environmental samples. He also suggested to include pooling protocols in validations and lyophilisates in the inter-laboratory study protocol. Moreover, John MARUGG informed that Nestlé was not in favour of naturally contaminated samples but would prefer particular artificial inoculation protocols.

Caroline de BACKER (University of LIEGE) is the representative of the Belgium National Reference Laboratory. She explained that the official list of authorized methods was published twice a year in Belgium. This list contains 3 types of methods : standardized methods, alternative validated methods and Belgium reference methods (when no other method exists)

The list was strongly modified by the Commission Regulation (EC) n° 2073/2005 so that from October 2006 to January 2008, all Belgium laboratories had to progress in order to comply with new requirements.

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In 2008 alternative validated methods are recognized by the Belgium Food Agency if they fulfil requirements of the Commission Regulation (EC) n° 2073/2005 and if they provide at least the same guarantees as those described in the protocol of the standard EN ISO 16140

As a conclusion to the discussions on food microbiology methods validation, **Paul in't VELD** (VWA, the Netherlands and Chairman of ISO/TC34/SC9/WG3) informed attending people on the future changes expected in Validation protocol EN ISO 16140.

WG3 is the international working group in charge of the EN ISO 16140 revision gathering 25 participants from 15 different countries/organisations Since the first meeting held in January 2006 two meetings per year were held so far.

The objectives of WG 3 are multiple so that tasks were divided into 6 project groups, each having a maximum of 6 participants dealing with the following subjects :

1. Establish terminology
2. Revision of EN ISO 16140 (full validation of **proprietary** methods)
3. Development of standard for in house validation
4. Laboratory verification of methods
5. Intermediate validation based on AOAC classification
6. Minimum requirements for establishing/revising reference methods

The work of project group 2 (PG2) is mainly based upon document of AFNOR Certification describing proposed corrections, modifications and interpretations to standard EN ISO 16140 (2003). Project leaders are Max Feinberg (INRA, France) and Paul in't Veld himself.

The topics discussed so far in order to revise the content of EN ISO 16140 dedicated to proprietary method validation are the following :

- Natural versus artificially contaminated samples
- Outline collaborative studies
- Statistics for the evaluation for qualitative data and quantitative data
- Inclusion of IDF 161 (Bactoscan) dealing with quantitative determination of bacteriological quality in milk

In the last 3 years significant progress has been made especially within PG 2 which will be the basis for the other PG's. It is intended to have the first draft from PG2 for discussion in WG 3 in September 2008. Depending on the discussion the draft can be discussed at the next ISO meeting in May/June 2009.

Paul in't VELD asked people to be patient and Bertrand LOMBARD recalled that standardization was a huge work involving many experts from a lot of countries at the international level, so that the time frame could not be as short as users and suppliers would like it to be.

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At last, **Frederic MARTINEZ** from BIO-RAD and **Bertrand COISSAC** from GeneSystems talked about their experience -as kit suppliers- in the certification of kits for detection/quantification of *Legionella* in water by PCR.

Frederic MARTINEZ showed the great need in *Legionella* monitoring for many types of water and in various installations. He explained that the PCR technique met the requirements for a fast *Legionella* monitoring as well as for driving installations and measuring the effectiveness of biocide treatments. PCR technique also enables to carry out quick research for contaminated sites in the context of epidemiological enquiry.

Bertrand COISSAC explained that in 2006 AFNOR had standardized method XP T90-471 for the detection/quantification of *Legionella* by PCR. Then AFNOR Certification has developed a validation protocol based upon this standard so that kits can be validated using this protocol since the end of 2006. In December 2007, the first kits were certified : GeneSystems *Legionella* and iQ-Check *Legionella* methods.

The certification using AFNOR protocol enables to validate the performances of the kits (LD, LQ, linearity, recovery, specificity). Moreover a ring trial is performed on artificially and naturally contaminated water samples in order to evaluate PCR reproductibility among more than 8 laboratories.

As a conclusion, Bertrand COISSAC explained that the use of an AFNOR validated protocol allowed to guarantee the PCR kit performances thanks to a third party validation. It is then easier for laboratories to gain accreditation for *Legionella* analysis by PCR.

