



Validation in feed and food microbiology : a unified approach set out by the Food Agencies or different approaches stimulated by the laboratories itself

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Introduction

Food microbiology is using a high number of analytic methods based on different principles and methodologies.

The implementation, on the 1st of January 2006 of the well known Commission Regulation (EC) n° 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs brought changes in the food microbiology sector by editing new requirements about the choice of analytic methods. The two main changes are on one hand, a list of reference methods and on another hand, the conditions to authorize the use of alternative validated methods. Those alternative methods have to be:

- validated compared to the reference method;
- in accordance with the protocol set out in the standard ISO 16140 or other internationally accepted similar protocols;
- certified by a third party.

Every European country has now to adapt and organise their laboratories in order to meet these requirements. In Belgium, this competence is assigned to the proper authority : the Federal Agency for the Safety of the Food Chain (FASFC).

In order to regulate and to give guidelines to the Belgian food microbiological laboratories even before the implementation of the Commission Regulation (EC) n° 2073/2005, the FASFC has set up a positive list of analytical methods that it recognises (appendix I). This list exists since about 11 years and is now updated twice a year. It contained many various methods but it is becoming limited since the implementation of the Regulation (EC) n° 2073/2005.

Analytical context

According to the analytical context in which are carried out the microbiological analyses, FASFC authorizes a more or less broad range of methods (table I).

Table I : requirements of FASFC for the choice of methods according to the analytical context

analytical context	FASFC requirements	in practice	date of application
R & D	no requirement		NA
private analyses	methods consistent with the Commission Regulation (EC) n° 2073/2005	any method among: - reference methods - alternative validated methods	01/01/08
FASFC analyses and all counter-analyses	methods imposed by FASFC among methods consistent with the Commission Regulation (EC) n° 2073/2005	imposed methods among : - reference methods - alternative validated methods	01/01/08

In the R&D sector, as long as the products are not put on the market and that the results are not used for the installation of HACCP plans, there is no requirement concerning the choice of the analytical methods.

Within the framework of the safety analytical controls carried out within the agro-alimentary companies, the methods used must be consistent with the Commission Regulation (EC) n° 2073/2005. This regulation is indeed intended to the operators of the food sector, without distinction. FASFC authorizes a last adaptation period until January 1, 2008, deadline. Other methods can also be used, but the results obtained by those are not taken into account at the time of the inspections and the audits.

In the frame of monitoring program organised by FASFC and counter-analyses, the choice of methods is more restricted. FASFC want to harmonize the system and will impose methods with the same performances among methods in agreement with the Commission Regulation (EC) n° 2073/2005 from 1st of January 2008 onwards.

In a general way, the idea is to follow the Commission Regulation (EC) n° 2073/2005 even in the private sector. This means that, if an alternative exists, national standards and Belgian official methods are progressively disappearing on the benefit of reference methods and alternative validated methods.

Methods categories

Methods in the list of the methods recognized by FASFC can be gathered in three categories (Tableau II) :

- standard methods ;
- alternative validated methods;
- Belgian official methods.

Table II : food microbiological analysis methods categories

method	category	examples
standardized	international	ISO, IDF
	european	EN
	national	NBN, NF, NEN, DIN, NMKL
alternative validated	ISO 16140	AFNOR, MicroVal?, AOAC?
Belgian official		SP-VG

The **standard methods** are described in a standard and are worked out or recognized by technical committees of standardization organizations. The activity of standardization take place on different levels :

- international : « ISO » standards issued from the International Standardization Organization (ISO), « IDF » standards issued from the International Dairy Federation (IDF) ;
- european : « EN » standards issued from the European Committee for Standardization (CEN) ;
- national : « NBN » standards issued from the Belgian Institute for Standardization (IBN) ; but also « NF » standards for France, « NEN » for the Netherlands, « DIN » for Germany, « NMKL » for Norway.

More and more, the standards are regularly updated and characterized in order to be able to indicate the performances that the laboratories must reach.

It is important that laboratories carry out the entire protocol of the standard and do not simplify it at all.

Since 1984, there is an obligation for the national institutes to transpose the European standards in national standards. Consequently, the European standards must be recognized by the proper authority, FASFC in Belgium.

French standard methods called "routine" (i.e. NF (or XP) V08-050 type and following) established by consensus of experts and who are used much by the Belgian laboratories, will not be validated against the reference method and will not fit with the Commission Regulation (EC) n° 2073/2005. The French Association of Standardization (AFNOR) decided their progressive abolition and FASFC fixed, for the Belgian laboratories, January 1, 2008 as a deadline. Other standards ISO or IDF, for example, are to be classified in the same category, but can still be used until January 1, 2008 (cf lists recognized methods, appendix 1).

The **alternative analysis methods** are commercial methods adapted to the requirements of an industrial production, which are simpler, faster and/or more economic than the traditional reference methods. The standard ISO 16140 of October 2003 entitled "Protocol for the validation of the alternative methods" was elaborate in order to validate these alternative methods compared to the reference methods. The protocol is rather heavy but if ever a success, brings an international recognition to the alternative concerned method.

In Belgium, we refer up to now to the certification of the French structure "AFAQ AFNOR Certification". **FASFC considers that the alternative methods validated by this independent certifier organization are in conformity with the provisions of article 5 of the Commission Regulation (EC) n° 2073/2005. The alternative methods validated by other organizations or laboratories will be also recognized in Belgium if they fulfill the requirements of article 5 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 ISO 16140.** The manufacturers or laboratories willing to use such methods are invited to provide the validation file of the method to FASFC and to the National Reference Laboratory in food microbiology for evaluation and possibly recognition.

In a general way, the food agency recognises since about 11 years standards and validated alternative methods, so Belgium doesn't have too much troubles to implement the new regulation.

The last category of method is the **Belgian official methods**. Those are worked out to meet a need when there are no alternative methods among the standardized methods or alternative validated methods. They are written by the National Reference Laboratory in food microbiology and recognized by FASFC. Those that do not answer this definition will be no longer recognized at January 1, 2008.

Available alternative methodologies

The alternative methods offer various advantages compared to the reference methods. They can present a greater speed of analysis and/or answer, a facility of execution and/or automation, or of the additional analytical characteristics (limit of detection, specificity, etc).

Principal methodologies are included in the table below (Table III).

Table III : principal available methodologies within the alternative validated methods and some examples

per culture		example of methods
counting	dehydrated media	pétrifilm
	chromogenic media	Rapid' media, media ID
	NPP	Tempo
detection	Culture mediums	SMS, ALOA, Rapid', CHROMagar, Compass, OCLA, OAA
	Immunoassay	Oxoid rapid test, Vidas, Transia, Tecra, Reveal, kit Locate, Bioline, Rapidyme
	Genetic tests	BAX, GeneDisc Cycler, IQ-Check, Probelia
	molecular hybridization	Lumiprobe, Accuprobe

To obtain more information on these methods, please refer to manufacturers.

Important note:

Within the framework of AFAQ AFNOR VALIDATION, the procedure of the alternative methods of pathogenic microorganisms detection (*Listeria*, *Salmonella*, *E coli* O157) must imperatively include a step of confirmation of the positive results.

This step can be issued from :

- a reference method;
- another AFNOR alternative validated method, but with another principle ;
- an original method proposed by the manufacturer and tested by AFAQ AFNOR Certification.

Conclusion

Since about 11 years the Belgian Federal Agency for the Safety of the Food Chain established a list of the analytical methods recommended for the food microbiological analyses in Belgium, in order to give guidelines to the laboratories. It highlights the reference methods indicated by the Commission Regulation (EC) n° 2073/2005 of 15 November 2005 and the methods recommended by FASFC within the framework of the analyses carried out by the approved laboratories.

This list evolves constantly, in particular since the implementation of the Commission Regulation (EC) n° 2073/2005 and of the adaptation period which is associated to it.

It is essential for the laboratories to regularly be interested in its evolution in order to anticipate the changes to keep the accreditation updated. A detailed attention is to be brought on:

- the imposition of the methods for the analyses for FASFC and the counter-analyses, as from January 1, 2008 ;
- the obligation for private analytical controls to also align on Commission Regulation (EC) n° 2073/2005, before January 1, 2008 ;
- the progressive disappearance of some standards such French standardized methods called "routine methods";
- the new possibilities that offer the alternative validated methods, methods recognized by FASFC if they fit to the requirements of article 5 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 ISO 16140.

The list of the recognized methods is updated approximately twice a year and is available on the website of FASFC (<http://www.afsca.be> > secteur professionnel > administration des laboratoires > agrément > laboratoires agréés > notes de service)

References and useful links

- Standard ISO 16140:2003, Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.
- Commission Regulation (EC) n° 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, December 22, 2005, L 338/1 – L 338/26.
- LECLERCQ A. (2003). EN ISO 16140, Validation des méthodes alternatives d'essais. Lecture during the 8th Conference on Food microbiology, Liège.
- <http://www.afsca.be> > secteur professionnel > administration des laboratoires : website of the Federal Agency for the Safety of the Food Chain (FASFC)
- <http://www.afnor.fr>: website of the French Association for Standardization (AFNOR)
- <http://www.wssn.net> : World Standards Services Network : network gathering websites of the international, regional or national organizations of standardization.