Experience in developing generic guidelines for method validation applicable in the areas of animal health, plant health, and food safety

Michel Laurentie

METHOD VALIDATION

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Abstract

A generic guideline for method validation was developed from all laboratories at the French agency for food, environmental and occupational health and safety (ANSES). This guideline takes into account the specificity of the various subject areas, such as animal health, plant health, or food safety. Furthermore it was developed for both qualitative and quantitative methods. A life cycle for analytical methods was also introduced. The process of validation was described according to the principle that the first step is to determine method performance using standard characteristics (trueness, precision, LOQ, sensitivity, and specificity) for qualitative or quantitative methods, and the second step is to compare the studied characteristics with the validation criteria defined in the tender specifications. This process concludes the validation of the analytical method. Estimating the uncertainty of results was also taken into account.

Keywords

- Guidelines
- Performance characteristics
- Validation criteria
- Validation process
- Uncertainty

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Introduction

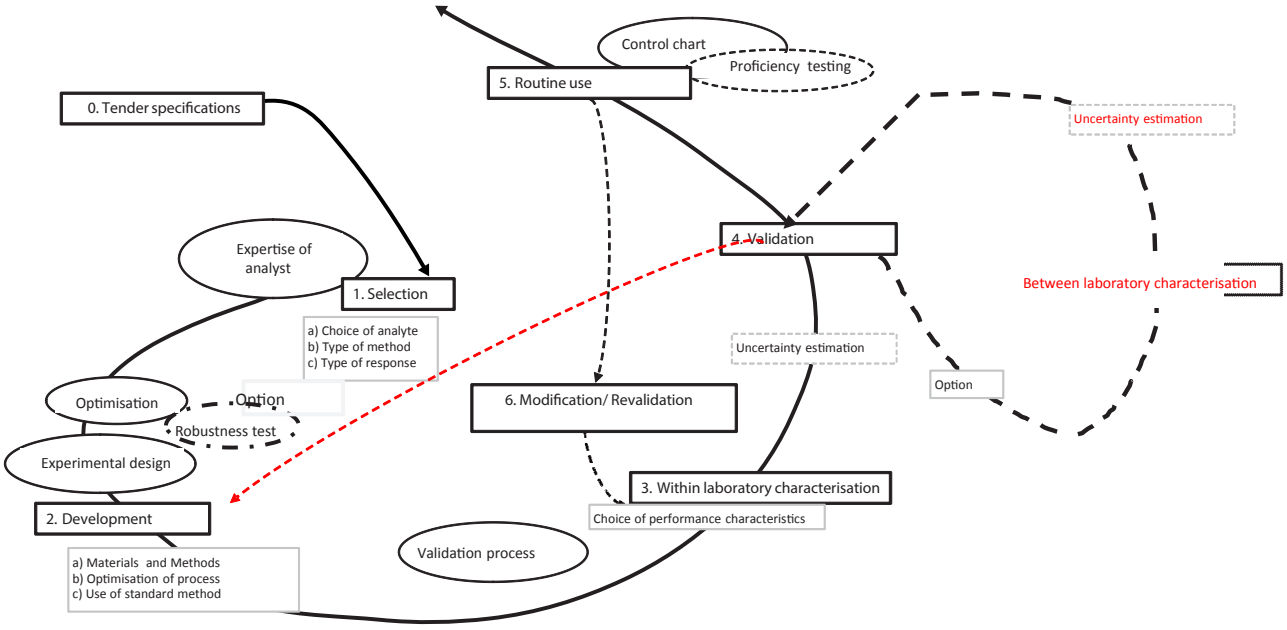
The French agency for food, environmental and occupational health & safety (ANSES) has many laboratories that develop multiple methods in different fields, such as animal health, plant health, and food safety. The majority of methods are in animal health (47%). For food safety and plant health, the percentages are 32% and 20%, respectively. Most of these laboratories are National Reference Laboratories (NRLs) or European Union Reference Laboratories (EURLs). Methods used at ANSES are generally standard methods (25%) and in-house methods (75%). These methods are developed and validated according to various national, European or international guidelines, which are generally specific to the area of interest. Furthermore, the provisions for methods of detection, quantification or confirmation are very different in different guidelines. However, standardisation and harmonisation of the overall validation process across laboratories is an important goal to achieve.

To attain this objective, a working group (WG) representing the range of expertise of laboratories was created. The skills included were: experience in qualitative (detection) and quantitative (confirmation, quantification) validation processes; experience in development and validation of methods in animal health, plant health, and food safety; quality management; and statistical approaches.

Since many ANSES laboratories are official control laboratories, they are accredited according to ISO/IEC 17025 [2015]. As a result, this standard was pivotal in establishing our internal guideline. In ISO/IEC 17025, validation is defined as “the confirmation by the examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled”. Consequently, the validation process is firstly the assessment of method performance, and secondly, the comparison with validation criteria to confirm that the methods are fit for the intended use.

At the first meeting, the WG established the roadmap to attain its objectives. It was also decided that it was necessary to have a general guideline to address the following requirements: the validation process should be harmonised, a report should be issued for the characterisation and validation of an analytical method, and guidance should be given on how to describe

FIGURE 1 / The different steps in the life cycle of analytical methods, including development, in-house validation, and reproducibility studies.



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an analytical method. This article will focus only on the general guideline.

Rapidly it became clear that there was a great deal of confusion regarding technical terms. For example, the term accuracy was confused with trueness. Linearity is also a misused term, since some analysts use the term linearity to describe the relationship between response and theoretical target (*e.g.* concentration or amount), while others use it to indicate the relationship between the calculated and theoretical target. In fact, the first is the response function and the second is true linearity.

Another term that is widely misused is sensitivity, sometimes understood as the quantification limit or the capacity of a quantitative method to detect a small variation, or the ratio of true positive results in qualitative methods.

It was therefore decided to develop a glossary. From the various guidelines, standards or official documents, 272 terms were collected, from which, after exclusion of synonyms, a glossary of 72 main terms was established. For each term, its definition was provided along with synonyms and the English translation. The main sources for the definitions were ISO Guide 99 [2007] and ISO 3534 [ISO 3534-1, 2006; ISO 3534-2, 2006].

The second step was to establish the life cycle of an analytical method [Feinberg, 2013] adapted to the objectives of French laboratories. This life cycle is presented in figure 1.

The WG clearly decided that development should not be included in the validation guideline, whereas the expression of the need should be clearly defined, as indicated by ISO/IEC 17025. As such, a chapter was specifically included in the guideline to indicate how to establish tender specifications.

The WG also decided, in compliance with official documents (*e.g.* the OIE Manual [OIE, 2014]) or other guidelines and standards, that the validation process should be performed by characteristics (trueness, precision, etc.) or by an overall approach (accuracy profile, total error) and these performances should be compared to validation criteria to determine the validity of the method. The WG defined the main characteristics to assess according to the type of analytical method (qualitative, quantitative) and the process of validation. Table 1 indicates these characteristics.

Table 1 / Main characteristics to assess depending of the type of analytical method.

Step in validation process	Characteristic		Characteristic of performance to assess depending on type of method	
			Qualitative	Quantitative
Characterisation within laboratory	Specificity [a, d]		X	X
	Sensitivity [b, e]		X	(X)
	Response function/efficacy (PCR)			X
	Precision	Repeatability	(X)	X
		Intermediate precision	X	X
	Trueness		Groundless	X
	Accuracy (trueness + precision)[c]		Groundless	X
	Linearity		Groundless	X
	Limit of	Quantification	Groundless	X
		Detection	X	(X)
	Range of validity		X	X

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Step in validation process	Characteristic	Characteristic of performance to assess depending on type of method	
		Qualitative	Quantitative
Characterisation between laboratories	Reproducibility	X	X
	Repeatability	(X)	X
	LOD	(X)	Groundless
	LOQ	Groundless	(X)
	Specificity a, d	X	Groundless
	Sensitivity b,e	X	Groundless
	Characteristics such as cost, time, ease of use, efficiency, etc. should be taken in account and indicated in tender specification	X	X

Groundless: characteristic is not relevant.

(x): characteristics in brackets are advised.

a: for qualitative methods, specificity may be analytical specificity or diagnostic specificity.

b: for qualitative methods, sensitivity may be analytical sensitivity or diagnostic sensitivity.

c: for quantitative analytical methods, accuracy is trueness and precision.

d: or false positives in some official documents.

e: or false negatives in some official documents

Certain other non-technical characteristics were also included. For example, the cost of a run, simplicity, ease of use, or duration of analysis are additional characteristics to be used to decide on method validity.

A statistical part was also developed to provide guidance on performing calculus. The main standards used were ISO 5725 [ISO 5725-1,-2, -3 and -4, 1994], ISO 3534 [ISO 3534-1 and -2, 2006] and ISO/FDIS 16140-2.2 [2016]. Annexes were also prepared to explain the statistical approaches, including basic statistics, such as how to verify normality, homogeneity of variance, estimated false positives or negatives, and why it is necessary to perform repetition to determine parameters. It was clearly explained that the performance of the method is described by characteristics (trueness, precision, accuracy) but assessed using statistical parameters (bias, standard deviation, etc.).

A specific part was included to describe how to estimate and use measurement uncertainty, based mainly on Guides JCGM 100 [2008] and ISO/TS 19036 [2006]. In addition, the possibility of estimating an uncertainty function [Gassner *et al.*, 2014] was reported.

Finally, a draft version was released to laboratories for comment and preliminary use. After a trial period of 2 months, about 200 comments were collected with 84% for the guideline. Some of these comments were about the text (45%) and others were on statistics or methodology (55%). The guideline was amended and corrected: 90% of comments were taken into account by the WG in two plenary meetings, and the final version is now currently used by laboratories.

It was also decided that it was necessary to have a “referent” for the validation of analytical methods in each laboratory and a national referent to coordinate the validation process and help analysts to use this guideline.

Finally, the process followed to establish the guideline is summarised in figure 2.

The WG worked collectively on certain general topics, such as tender specification, but also in smaller task groups for specific parts, including qualitative or quantitative methods.

In conclusion, the guideline and associated documents were established in about 20 months.

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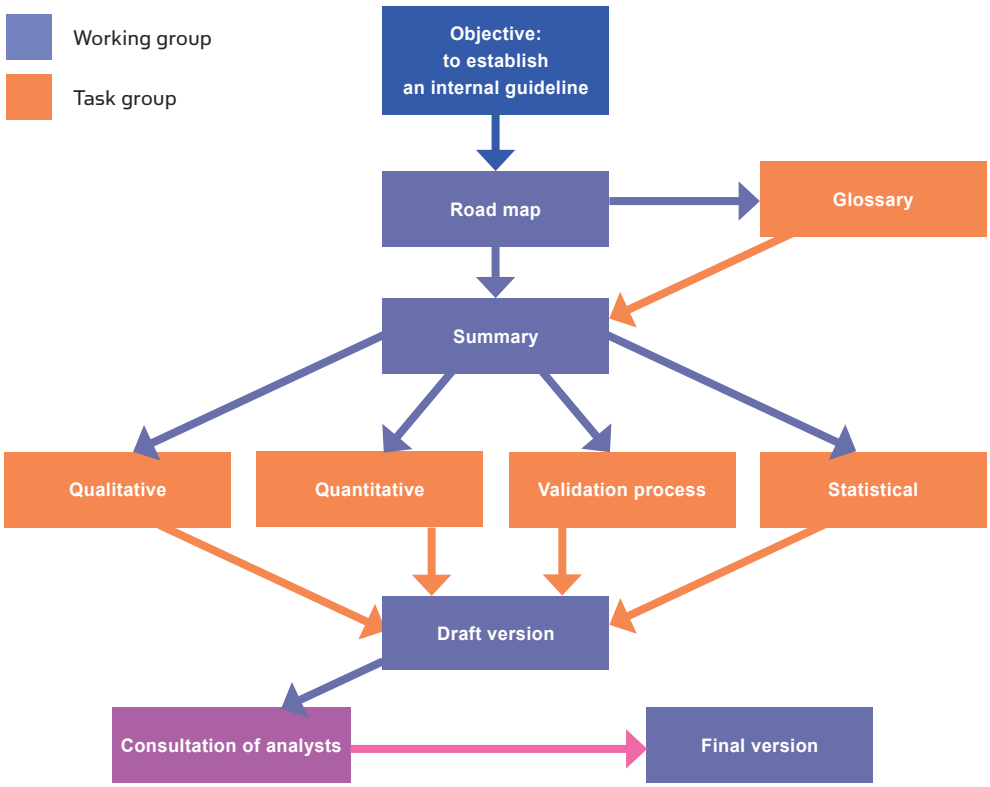
FIGURE 2 / Process to establish the guideline.



It can now be used in the various areas of interest of ANSES laboratories. This guideline does not aim to replace specific guidelines, nor official documents in animal health, plant health, or food safety, but enables harmonisation of the validation process across laboratories. The guideline also focused on the critical points in validating methods: establishing tender specifications, defining and assessing characteristics of performance, developing validation criteria, and estimating uncertainty.

(Guideline in French available at: https://www.anses.fr/fr/system/files/ANSES_GuideValidation.pdf).

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