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French procedure for the formalisation of analytical methods in the area of plant health

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The purpose of this article is to present the procedure followed in France to develop and validate official methods in the area of plant health. It was established jointly by the supervisory ministry (Agriculture) and the National Reference Laboratory (ANSES Plant Health Laboratory) in order to take into account each partner's constraints and objectives. While the procedure remains open to change, specifically so that new method characterisation approaches and new techniques can be integrated, it is now organised around several major phases, each of which is presented below. One of the unique features of this procedure, specific to the field of plant health, is its transparency, which is ensured by external consultation.

Introduction

To protect the national territory against quarantine pests and ensure corresponding surveillance activities in accordance with the EU regulatory provisions in force (specifically Directive 2000/29/EC and its implementation texts), the French State implements surveillance and control plans. Surveillance plans apply to plants and plant products upon import, as well as those already present on the national territory (in nurseries, in the field, etc.). To guarantee the quality of exported products, analyses can also be carried out in the framework of EU plant passports, or with the aim of issuing health certificates for non-EU countries.

The analyses carried out on behalf of government bodies, such as the Directorate General for Food (DGAL), Regional Food Authorities (SRAL) and the Border veterinary and plant health inspection service (SIVEP), are termed "official" analyses. Aside from certain specific cases, these analyses can only be performed by accredited laboratories, National Reference Laboratories (NRLs), or so-called "recognised" laboratories (French Rural and Maritime Fishing Code (CRPM), Article R. 202-8). As the advocate, the Directorate General for Food defines the methods that are to be used for these analyses (Article R. 202-17 of the CRPM). Although the use of alternative methods is possible under the provisions of this article, use of the official methods ensures consistency in the surveillance system and reliability of results supplied by the 20 accredited laboratories that are part of this network in France (see list at the following address (in French): http://agriculture.gouv.fr/la-liste-des-laboratoires-agrees).

The purpose of this article is to present the formalisation procedure for analytical methods in the area of plant health, as it is currently implemented by the ANSES Plant Health Laboratory and the DGAL within the Ministry of Agriculture, Food and Fisheries.

Overall presentation

The definitions of the terms used in this article that serve as a framework for the Plant Health Laboratory are presented in Box 1 - General definitions concerning methods, and in Box 2 – Definitions concerning method performance criteria. The full process for formalising a method in the field of plant health is shown in Figure 1. The four main phases can be summarised as follows:

- determination of requirements, method selection and development;
- method characterisation and intra-laboratory validation (sometimes on an inter-laboratory basis, when needed);
- external consultation for the draft method, including public consultation;
- method formalisation by the competent authority.

These phases are presented below with a focus on the particular features or specificities of plant health compared to other fields of activity.

Determination of requirements, method selection and development

Method development and scientific and technical support to the supervisory body are among the specific missions of National Reference Laboratories, as indicated in the CRPM, Article R. 202-5. As such, the methodological needs for the official analytical purposes of the State are conveyed to the NRL.

Given that official methods must be suitable for their intended use in order to be validated (see below), the preliminary discussions between the NRL and the sponsor are a key phase for the success of any project. During this phase, it is essential that the explicit and implicit requirements of the client, for example the DGAL, be clearly determined.

In the area of plant health, the DGAL's general needs are laid down in a document called "specifications for the validation of official analytical methods", signed jointly by the DGAL and the NRL, which acts as a service provider for method selection and development. While the specifications can be adapted to each case, depending on the specific pest involved, the epidemiological background, the degree of urgency, etc., they form a general framework specifying the criteria for selecting a method depending on its intended use. For example:

- an analytical method intended to support management of an outbreak will be more suitable for its purpose if it provides rapid, cost-effective results. The aim in this case is to obtain results for a large number of samples in a short period of time, in order to delineate the area of infection;
- a method intended for the control of imported plant products to detect a quarantine pest that is not present on the national territory will need to be as sensitive as possible to avoid introducing any such quarantine pest, and will need to provide fairly rapid results to enable batch release of the consignment.

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Analytical method
Written procedure describing all the means and operating conditions required to detect and/or [identify] [...] the analyte, including: scope, principle and/or reactions, definitions, reagents, equipment, operating procedures, expression of results, precision, and test report.

Alternative analytical method
Analytical method used by a laboratory instead of a reference analytical method.

Reference analytical method
Analytical method recognised by experts or used as a reference by agreement of the parties that yields, or is assumed to yield, the accepted reference value for the physical quantity of the analyte to be measured.

Official method
Analytical method drafted by the NRL and published in the Official Bulletin of the Ministry of Agriculture, to be used when performing official analyses.

Method evaluation (= characterisation of method performance criteria)
Determination of the values of the performance criteria of the method.

Box 1. General definitions concerning methods

These initial discussions between the DGAL and the NRL therefore aim to lay down specific objectives based on the expected target performance criteria, mainly concerning theoretically acceptable levels of false negatives (sensitivity) and false positives (specificity). However, as the examples above demonstrate, criteria other than technical performance parameters (such as rapidity, costs, timelines, and ease of use) must also be taken into account when defining the suitability of a method for an intended use. The purpose may indeed prompt the NRL to opt for one method over another, particularly since choices often need to be made, and a balance struck between sensitivity and specificity criteria.
Once the objectives and expectations have been defined, the reference laboratory carries out a literature study to determine the state of the art, and then, i) develops a method in-house, or ii) performs an initial comparison of existing methods (scientific publications, etc.), or iii) outsources method development. Once these activities have been completed, the laboratory must have a method that can then be characterised in terms of performance criteria. It should be noted that some data collected during the development phase may serve as a basis for the characterisation report.

Method characterisation and (intra-laboratory) validation

A number of standards propose method characterisation methodologies. Some are relatively general (ISO 16140, ISO 5725, etc.), while others are more technical and specific to the area of plant health (EPPO PM7/98). On the basis of these standards and the specifications established with the DGAL,

Sensitivity (of a method)
Probability of detecting a target organism (positive result) in an infected or contaminated test substance. In other words, the ability of a method to detect the analyte when it is present in the sample.
The concept of sensitivity includes inclusivity and detectability (or analytical sensitivity):
- inclusivity: Ability of the alternative method to detect the target analyte among a large range of strains. It can be expressed as a percentage of detected strains or by the known risk (given the state of knowledge at the time of testing) related to evaluation of target intra-taxon variability;
- detectability: Ability of an alternative method to detect the target analyte in a serial dilution.

Specificity
The degree to which an analytical method concerns only the property or analyte of interest, with the certainty that the result is derived only from the analyte.
In other words, specificity is:
the ability of the method not to detect the analyte when it is not present in the sample;
or the ability of the test to provide a negative result for a healthy sample.
Note: specificity is basically the same as exclusivity: Absence of interference by a suitable range of stains, isolates, populations, etc. that are not targets of the method.

Accuracy
Closeness of agreement between a test result and the accepted reference value. In other words, the number of agreements between the results obtained and those expected, relative to the total number of results.
It includes both the sensitivity and specificity of the method.

Detection limit or threshold
"The lowest concentration or amount of analyte that can be detected [...] in the experimental conditions described in the method". It corresponds to analytical sensitivity.

Repeatability
Closeness of agreement between successive and independent results obtained with the same method, for the same test material, in the same conditions, i.e. equipment, operator, and laboratory, within short intervals of time (repeatability conditions).

Reproducibility
Closeness of agreement between individual test results obtained with the same method, for the same test material, by operators in different laboratories, using different equipment (reproducibility conditions).
A reproducibility test involves analysing the same sample in different conditions. In this case, the coefficient of variation is a simplified expression of the reproducibility of the method.

Box 2. Definitions concerning method performance criteria

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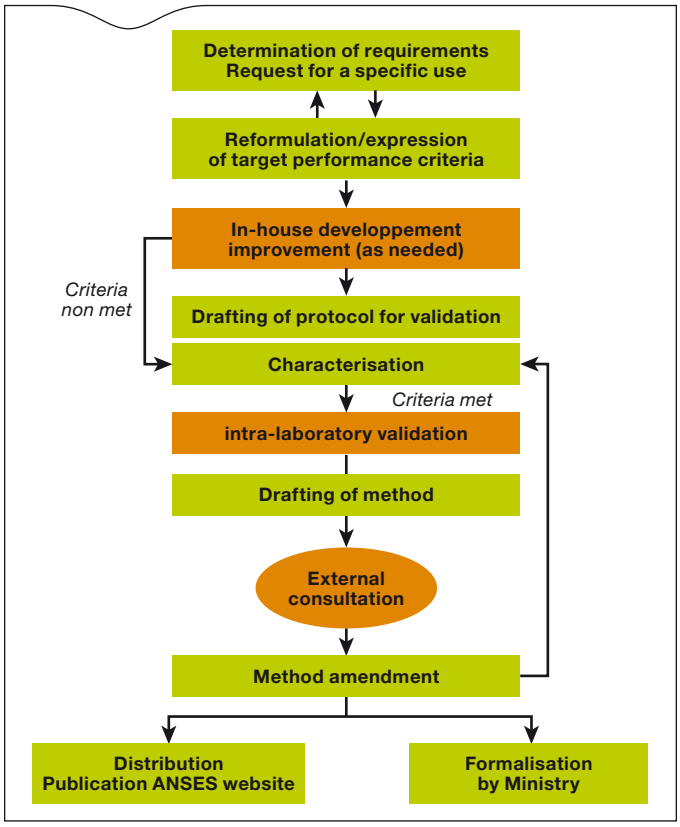

Figure 1. Process for preparing and formalising methods in plant health

the Plant Health Laboratory has drawn up an in-house guide on characterisation of method performance criteria.
Generally, methods used by the NRL or intended for formalisation undergo characterisation of the following technical performance criteria (see Definitions, Box 2):
- sensitivity (in terms of inclusivity), specificity and accuracy;
- detection limits;
- repeatability;
- intermediate precision (intra-laboratory reproducibility).

Other non-technical criteria such as cost, ease of use and so on, are evaluated on a case-by-case basis.
At the start of 2013, revision of this guide prompted the laboratory to:
- introduce a calculation for uncertainty regarding the sensitivity, specificity and accuracy parameters;
- make provisions for studies on robustness with minor but deliberate variations in parameters that are important for the overall reliability of results, for the specific case of methods that are to be delegated.

The full results of characterisation testing are compiled in a report. These data are then compared with the predetermined target performance criteria to decide on the degree to which the method meets its intended use.
- if the target criteria defined by the client cannot be fulfilled due to technical limitations:
  - work is carried out to optimise the method or develop a new method,
  - or the specifications are amended by the client,
  - or implementation of combined methods and/or restricted conditions in which the methods can be used;
- if the target performance criteria are met, the method:
  - can be validated if it is to be used in-house by the NRL;
  - is submitted for external consultation if it is to be delegated to a network of accredited laboratories.

Ultimately, this intra-laboratory characterisation of performance criteria is very similar to the process that may be followed in the other areas of expertise within ANSES, such as animal health or food safety. However, a moderate number of samples are generally tested (depending on the pest of interest) compared to other areas, due to the low number of available naturally-infected samples. This is particularly true for pests that cannot be cultivated or that are difficult to maintain in reference collections.

External consultation

In agreement with the Ministry of Agriculture, the Plant Health Laboratory has included an external consultation phase in the method validation process, including:
- scientific peer review;
- public consultation.

Peer review is carried out at least for all methods intended for delegation, but may also be extended to methods used by the NRL. It is generally conducted by two French-speaking experts in the corresponding field of study.
Public consultation involves publication of draft methods on the Agency's website (French only - http://www.anses.fr/fr/content/m%C3%A9thodes-danalyse-dans-le-domaine-de-la-sant%C3%A9-v%C3%A9g%C3%A9tale), possibly after amendment further to the peer review process. Consultation is usually open for a period of two months. The aim of this phase is to obtain comments from the public, at least from future users, to identify potential implementation issues concerning the draft method from a technical point of view, or to obtain information on how well the operating procedures are understood.
The comments received are then used to draw up a final version of the operating procedure that takes account of different approaches to facilitate implementation and transfer to laboratories other than the one that developed and characterised the method performance criteria.
Although the public consultation process for draft methods is directly based on existing administrative or standardisation procedures, it is unique and specific to plant health among the various sectors in France involved in development of official methods.

Formalisation

As mentioned above, a method can only become official if the DGAL, as the risk management authority, indicates in writing that the method is to be used for official analyses.
As a result, once the final version is drafted following public consultation, the NRL submits the method to the DGAL along with all the data used to obtain its validation, specifically the performance criteria. On the basis of the submitted data, the DGAL can then formalise the method, unless the background context has changed or there are additional specifications.
In the past, formalisation of methods required a notice to laboratory heads to be published in the Official Journal of the French Republic. Some official methods that have not yet been revised according to the current process have still not been

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amended in the Official Journal. Formalisation now involves publication of an administrative notice 'note de service' by the DGAL that are made available to users, and more widely to the public, in the Official Bulletin of the Ministry of Agriculture (French only - http://agriculture.gouv.fr/bulletin-officiel). These notices specify in particular the methods' conditions of use (import, surveillance, etc.).
The methods themselves, i.e. the technical operating procedures, are made available at no cost to accredited laboratories and to the general public via the ANSES website (French only - http://www.anses.fr/fr/content/m%C3%A9thodes-danalyse-dans-le-domaine-de-la-sant%C3%A9-v%C3%A9g%C3%A9tale).

Conclusion

The procedure for formalising analytical methods in France in the area of plant health has been developed gradually by the National Reference Laboratory and the risk management authority. These interactions between the sponsor and service provider have helped to develop a framework that covers both the needs of the DGAL in terms of reliability and standardisation of analytical test results, and the needs of the NRL in terms of determination of expectations and accreditation requirements (so-called recognised methods). This model includes phases that are currently different from those in other areas of expertise within ANSES, specifically dialogue with future users via public consultations, and constitutes an interesting alternative to the conventional standardisation process.

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