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Development of detection methods within the framework of a flexible scope accreditation in accordance with the ISO/IEC 17025 Standard: experience at the French Plant Health Laboratory

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Abstract

In its role as National Reference Laboratory, the Mycology Unit of the ANSES Plant Health Laboratory is tasked to develop and validate detection and identification methods for phytopathogenic fungi.

In 2014, the laboratory obtained an extension of its accreditation in accordance with the ISO/IEC 17025 Standard to use methods that are developed and validated in-house, within a flexible scope framework. Most of these methods are based on molecular biology techniques.

This article presents the various actions implemented to develop and validate new detection methods under accreditation, and the adjustments that the laboratory made to its quality management system to integrate this methodological activity.

Keywords

- Flexible scope framework
- ISO/IEC 17025:2005 Standard
- Method validation
- Plant pests
- Quality management system

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Introduction

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has 11 reference and research laboratories, including the Plant Health Laboratory. This laboratory is made up of six technical units specialised in the detection and identification of organisms harmful to plants. The Mycology Unit located in Malzéville is the National Reference Laboratory (NRL) for the detection and identification of phytopathogenic fungi.

The Mycology Unit's main mission is to develop and validate specific detection tools for phytopathogenic fungi or oomycetes of interest potentially posing a risk to the national territory, that are officially regulated or included in quarantine lists, and that are emerging in France or other countries. Requests for development may come from external sources, relate to the unit's NRL mandate, or be initiated directly by the laboratory itself. Most of the tools developed are based on molecular biology techniques. The methods developed by ANSES can then be transferred to accredited laboratories to carry out routine analyses. As a result, the unit tries to develop detection tools making use of real-time PCR techniques that have higher performance and are far easier to standardise.

The Mycology Unit of the Plant Health Laboratory is accredited by the French Accreditation Committee (COFRAC) in accordance with the ISO/IEC 17025:2005 Standard for detection and identification analyses of phytopathogenic fungi and oomycetes. In 2014, the Mycology Unit decided to apply for an extension to its accreditation to use methods that it had developed and validated in-house, within a flexible scope framework. To do this, the laboratory made use of the quality management system it developed to carry out analyses, to which it added a chapter specifically on the implementation and traceability of new method development and validation.

Developing new detection methods under accreditation

■ Ensuring the admissibility of the request

The requester sends the specifications presenting the parasite of interest, the plant matrix (fruit, twig, seed, etc.) and the intended objectives on the basis of the implementation context, i.e. rapid method for border controls, low-cost method for serial analyses, or higher sensitivity than the reference method.

The laboratory examines the admissibility of the project on receipt of the request. This study takes into account a variety of criteria such as the conditions of implementation depending on the availability of the biological materials (*e.g.* pests not present in the European Union), limiting factors (*e.g.* obligate biotrophic species), the actions required (training, equipment purchases, etc.), and also constraints in terms of personnel availability. The laboratory also evaluates how demanding the request is concerning various criteria (*e.g.* complete absence of false positives).

The head of the unit then decides on the admissibility of the project. The project may be admissible, provisionally admissible (need for funding, establishment of a partnership, etc.) or not admissible.

■ Describing the project precisely

The development of new methods begins with establishing the current state of knowledge. This step involves documenting the various scientific techniques or approaches related to

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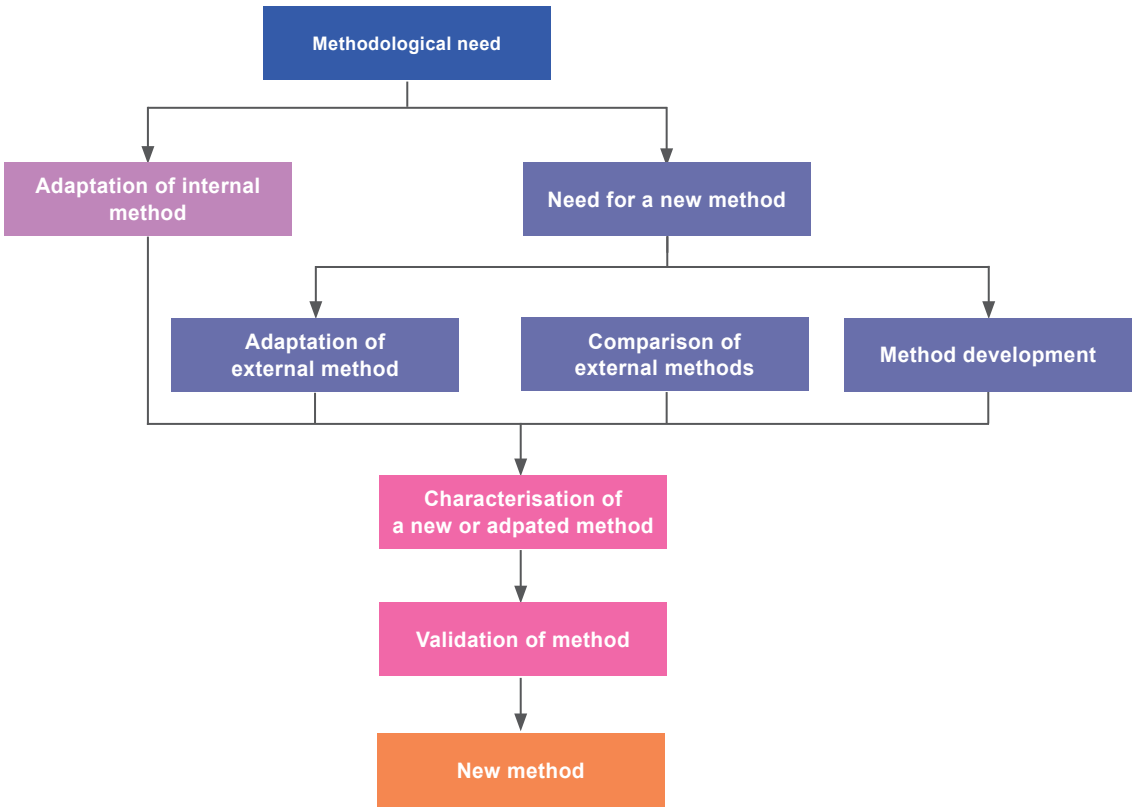
the project. For each of the publications, the advantages and disadvantages are described in detail. Analysis of these data makes it possible to define the best possible approach or approaches to meet the project objectives. The four main approaches retained by the laboratory are: adaptation of an In-house method, adaptation of an external method, comparison of external methods, and lastly development and optimisation of a new method. The analysis completed on the basis of the current state of knowledge enables the project leader to describe the project precisely and to select the various steps required to develop a new method (Figure 1).

FIGURE 1/ Summary diagram of the various steps required to develop a method on the basis of the selected approach.



Adaptation of an in-house method

Adaptation of an in-house method involves changing a method that has already been developed and validated by the Mycology Unit to adjust it to the needs of the laboratory or client. Method adaptation is required when changing a reagent, consumables, experimental parameters, or critical equipment. It includes a simplified characterisation step and a validation step.

For each change that can be made to the method, performance criteria to be re-characterised and practical aspects to be followed are defined by the laboratory.

Adaptation of an external method

Adaptation of an external method occurs when, following assessment of the current state of knowledge, it appears that only one method corresponding to the request is available publicly (scientific literature, international protocol, etc.), and that it can be used to develop the new

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method. Performance criteria of this adapted method are characterised and validated.

Comparison of external methods

Comparisons of methods are carried out when various protocols that fulfil the requirements of the request are already available in the scientific literature. Following an assessment of the current state of knowledge, the project leader selects at least two methods available in the scientific literature that in principle respond to the request. Comparisons of methods involve evaluating and comparing several performance criteria, as a first step, in order to retain only the protocol that is most suitable, in view of its complete characterisation and validation.

Method development

A new method is developed when no satisfactory method in terms of the request is available in the scientific literature or in international protocols. Method development is the process of designing and optimising the various steps in the method in which the most important physical, chemical, and biological parameters are evaluated and adjusted to suit the intended application of the method (adaptation to the matrix, to the analyte, or to the practical conditions in which the method will be used).

■ Characterising the method's performance criteria

In most cases, the methods already described in the literature use equipment, reagents, and consumables that are completely or partially different to those commonly used in the laboratory. Except in exceptional cases, such as a reagent or equipment indicated in publications as mandatory, adaptation of an external method will be carried out using reagents, consumables, and equipment similar to those in the original protocol, but available and commonly used in the laboratory.

The project leader selects the performance criteria to characterise, following the assessment of current knowledge. He or she defines the way they are characterised: expected performance values, statistical tests required, and types of samples to test, etc. In the framework of laboratory activities, the method is characterised by evaluating the various performance criteria presented in Table 1 (non-exhaustive list). According to our procedure, robustness is always characterised; the other optional criteria are selected on the basis of the specifications.

For each of these criteria, the laboratory has described how they will be evaluated (*e.g.* use of DNA extracts at standardised concentrations to determine analytical specificity) and defined the expected limit values (*e.g.* the reproducibility of the method must be greater than or equal to 80%).

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TABLE 1 / Non-exhaustive list of the performance criteria to characterise.

Mandatory criteria *	Evaluation of the efficacy of a PCR reaction	
	Analytical sensitivity:	Determination of the smallest detectable quantity of the target that it is possible to measure with a defined certainty.
	Inclusivity:	Ability of the method to detect the target taxon regardless of geographical origin and host, etc.
	Analytical specificity:	Ability of the test to provide a negative result for a non-target organism.
	Repeatability:	Consistency between successive and independent results obtained with the same method and using an identical test sample in identical conditions.
	Reproducibility:	Consistency between results of individual tests performed on an identical test sample and using the same method obtained by operators using different equipment.
	Diagnostic sensitivity:	Proportion of infested or infected samples yielding a positive result with the test of interest.
	Diagnostic specificity:	Ability of the test to provide a negative result for a healthy sample.
Optional criteria *	Robustness:	Ability of the method to remain unaffected by small deliberate variations in the experimental parameters described in the method.
	Evaluation of the quality of DNA extraction by an external (monoplex) or internal (multiplex) real-time PCR test targeting the 18S gene.	
	Ability of the test to be used in multiplex, i.e. to be used in parallel with other PCR tests in real time in the same reaction tube (*e.g.* test for another target, internal control of DNA extraction, etc.).	
	Evaluation of the minimum number of test samples to be used.	
	Ease of use and transfer.	
	Estimate of all the costs generated to produce the results: personnel, infrastructure, liquids, consumables, reagents, etc.	

*According to ANSES Generic guidelines for method validation.

■ Validating the new method

The ISO/IEC 17025 Standard indicates that “validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled”.

Validation corresponds to recognition of the ability of the method to meet the intended use. It involves comparing the values for the performance criteria with the expected values for the method. The validation phase must also confirm through tangible evidence that the level of method performance complies with the requester’s specifications (e.g. cost and duration of analysis) (Table 2).

The new method is considered suitable for the intended use, and validated, if the values for the performance criteria as described in the description of the projects are achieved.

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TABLE 2 / Example of validation of performance criteria. Extract from the validation report of the method for detection of *Plasmopara halstedii* by real-time PCR (Ioos *et al.*, 2012).

<table>
<tr><th>Performance critera</th><th>Results obtained</th><th>Expected results</th><th>Validation decision</th></tr>
<tr><td>Characteristics of the real- time PCR reaction</td><td>The effectiveness of the reaction is evaluated at 1.20 for monoplex reactions, and at 0.87 for duplex reactions. The detection threshold of the target is not affected by the duplex reaction from a qualitative point of view when calibrated plasmid solutions are tested because the detection limit remains the same. However, from a quantitative point of view, a mean lag of about 3 cycles is obtained at the detection limit. Ultimately, it was demonstrated that the mean Ct value obtained from DNA extracts of contaminated sunflower seeds (N=27) was not significantly different between a qPHAL monoplex test and a qPHAL + 18S uni duplex test (F=1.02; p=0.320). The R2 calculated for a monoplex reaction in a diluent of ultrapure water is 0.99</td><td>Duplex reaction as effective as monoplex reaction 0.80<E<1.20
R2 ≥0.98</td><td>Use in duplex format possible

OK</td></tr>
<tr><td>Repeatability and reproducibility</td><td>
<table>
<tr><th>Target</th><th>Target concentration[a] (number of plasmid copies in the PCR tube)</th><th colspan="2">CV (%)</th></tr>
<tr><th></th><th></th><th>intra-assay</th><th>inter-assay</th></tr>
<tr><td rowspan="3">P. halstedii qPHAL-F/-R PCR product</td><td>2.26 10^4</td><td>0.45</td><td>2.21</td></tr>
<tr><td>2.26 10^3</td><td>0.52</td><td>1.52</td></tr>
<tr><td>2.26 10^2 [b]</td><td>1.98</td><td>1.69</td></tr>
<tr><td>P. halstedii DNA</td><td>n.d.[c]</td><td>1.74</td><td>4.04</td></tr>
</table>
[a] Plasmids in which was inserted the qPHAL-F/-R region, diluted in a background of H.annuus DNA.
[b] This concentration was determined as 10 times the limit of detection of the test.
[c] Total DNA extract from a naturally infected H. annuus seed sample (02 FU)

The qualitative repeatability and the quantitative reproducibility are both 100%</td><td>Reproducibility and repeatability >80%
Coefficients of variation<10%</td><td>OK</td></tr>
<tr><td>Other criteria
Duration of analysis</td><td>Estimated duration of new method: 1 day
Duration of former method MH/07/24: 2 days</td><td>At least as short as the reference method</td><td>OK</td></tr>
</table>

A development and validation dossier is prepared for each new validated method. This dossier contains the following information:

- the requester's specifications,
- analysis of the current state of knowledge,
- a description of the methodological project,
- records of characterisation and, if necessary, optimisation,
- documents related to thought processes, all planned protocols, tests and raw data,
- handling sheets,
- validation report,
- etc.

Adapting the management system to method development

The laboratory decided to make use of the existing quality management system to perform PCR or real-time PCR detection analyses. The tests used for method development most often require the same resources (facilities, equipment, etc.). However, when necessary, specific new provisions for method development were implemented to meet the requirements of the ISO/IEC 17025 Standard.

■ Personnel

The laboratory personnel are in charge of method development, and if needed, trainees are recruited specifically for this project. Qualification and maintenance of operator competence

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is ensured by regularly conducting detection analyses that rely on molecular biology techniques. These analyses performed under accreditation are subject to regular audits, during which the competence of operators is assessed.

Integration of this new activity in the quality management system required the creation of a new key function called “project leader”. Qualification criteria based on initial training and professional experiences have been defined. This competence is evaluated annually.

The project leader’s role is to formalise the project, manage and follow up its implementation, and ensure the validation of the method.

Equipment and consumables

The material used in methodological development projects is the same as that used in analyses under accreditation. All the critical equipment is assessed in terms of metrology.

Plastic consumables, including tubes, microtubes, PCR tube strips or real-time PCR tube strips, pipette microtips, etc., are, as far as possible, the same as those used in the context of analyses under accreditation.

Reagents

The reagents used (enzymes, master-mix, buffers, etc.) are, as far as possible, the same as those used in analyses under accreditation. However, the project may aim to test and evaluate new reagents that are not yet used by the laboratory. In this case, like for reagents already used by the laboratory, batches are tracked and used in accordance with the manufacturer’s recommendations in terms of preparation and storage. Procedures of the quality management system for the purchase, reception and suppliers evaluation apply for these new reagents.

Project review

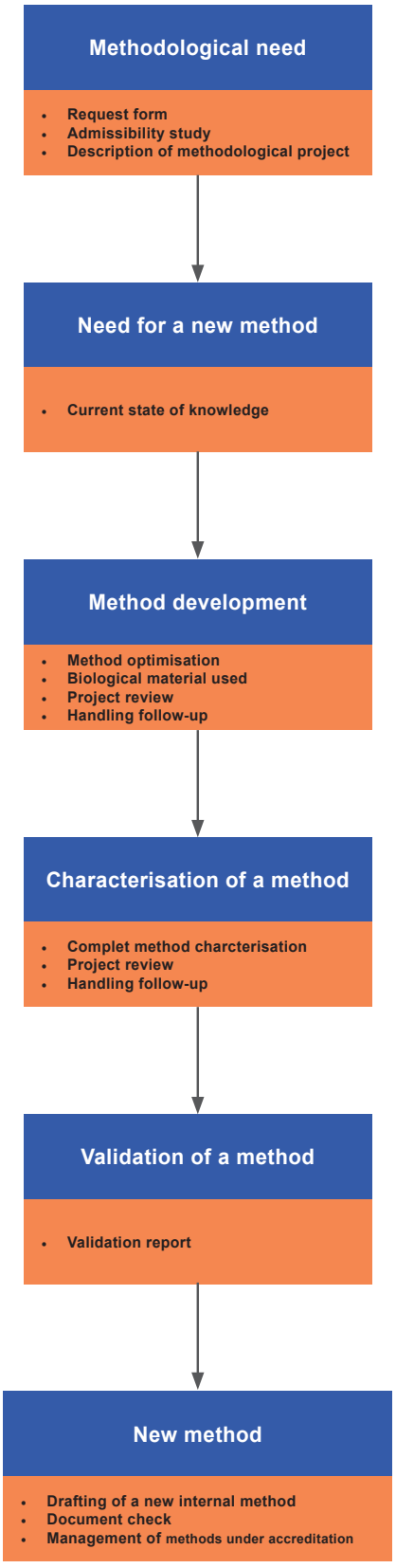
The project leader and operators carry out one or more reviews during the project. The reviews are aimed at evaluating the conduct of the project with regard to the initial project plan or its revisions. At each method development phase, the project leader can decide to revise the initial choices if necessary. Review is also required when results are not those that were expected. Project review is also a regular milestone helping to ensure traceability of activities and to check that no documents are missing. Lastly, it is a chance for the members of the team to exchange opinions. Each project review gives rise to a summary report used to track conclusions and the decisions made.

Traceability and data management

Traceability must ensure that required information is available to reproduce all or part of the results obtained during method optimisation and characterisation of the performance criteria. Records must also provide proof that resources used (reagents, equipment, operators, etc.) are suited to the task.

A unique feature of the project is the traceability method chosen by the laboratory. New forms are not only used for the traceability but they also provide a checklist for operators and project leaders. All the forms to fill are

FIGURE 2 / New specific forms to fill for each step of method development.



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ready to use with specific empty fields. The objective is to ensure that all the project leaders work in the same way and more importantly, that no criterion is omitted during method optimisation or characterisation of the performance criteria. As such, any failure to perform a step (or failure to characterise a criterion) must systematically be justified.

Detailed forms have been developed for each step. To guide the project leader, a summary diagram of all the specific forms for method development has been included in the project description sheet (Figure 2).

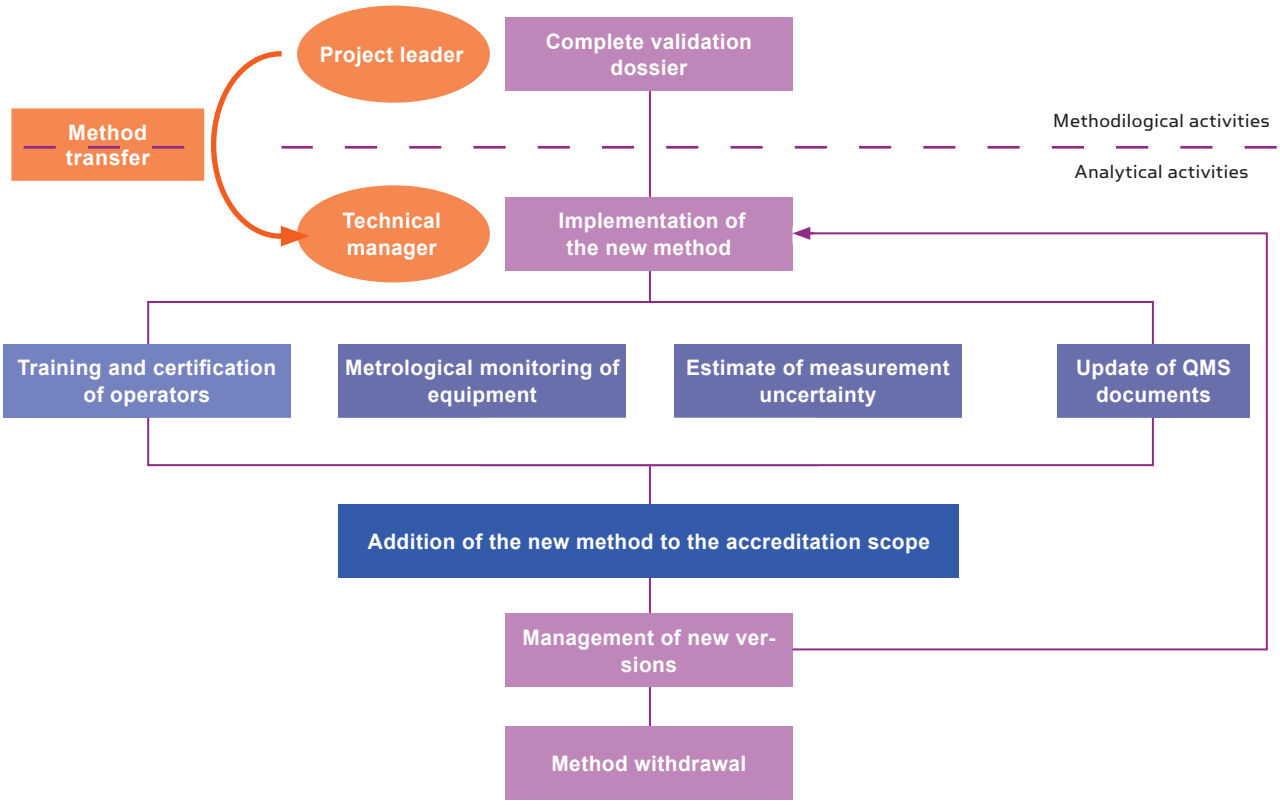
■ Integrating the method in the flexible scope accreditation

Once the validation report is complete, quality manager carries out a document check on the entire dossier of the new method or on the items specific to adaptation of an in-house method. Any technical or organisational deviation observed, and the corresponding corrective measures taken, are governed by procedures for non-compliance with provisions and non-compliant work.

Once the dossier is considered complete, the method can be implemented to carry out analyses in the context of flexible scope accreditation.

The project leader forwards the method to the technical manager in charge of detection analyses using the method. This person trains and certifies operators, estimates measurement uncertainty, and drafts quality documents required for traceability of analyses (Figure 3). For the Mycology Unit at the Plant Health Laboratory, the qualification phase of the technical manager and the operators is facilitated by the fact that they have most often participated in method development.

FIGURE 3 / Addition of a new method to the accreditation scope presented (type B flexible scope)



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Conclusion

The extension of the quality management system to methodological activities made it possible to define precisely the various steps in method development for the detection of phytopathogenic fungi, and to implement effective traceability.

The main difficulty encountered in developing the procedure to describe this process was to establish a list of the various types of cases found in method development for plant diseases that was as exhaustive as possible. Applying this procedure then required the creation of many record forms. Since traceability is often considered a very time-consuming constraint, most of the forms were developed to assist in writing. As such, for each step, the form was designed to be as exhaustive as possible.

This procedure and the associated forms provide practical assistance to project leaders and operators but require caution in terms of regular review.

In addition, because of this traceability, the project leader has all the necessary information to draft a scientific publication. As the project moves forward, all the characterisation and validation data, and all the metrological guarantees, are recorded. A corresponding, peer-reviewed scientific publication helps to demonstrate the value of the project.

Flexible scope accreditation enables the Mycology Unit of the Plant Health Laboratory to integrate methods or withdraw them from its scope depending on its needs as a National Reference Laboratory, for instance in health crisis situations or for emerging parasites. In this way, the laboratory can quickly respond to requests, while maintaining a quality management system that fulfils the requirements of the ISO/IEC 17025 Standard and that is suitable for the unit's size.

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