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Flexible scope experience Anses-Plant Health Laboratory Mycology Unit France

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Workshop on Flexible Scope - Wageningen, 2017-06-26/28

Anses - Plant Health Laboratory
Mycology Unit

Nancy / Malzeville

- Mycology Unit is the National Reference laboratory (NLR) for the detection and identification of phytopathogenic fungi

- Accredited since 2006 by the French Accreditation Committee (COFRAC) in accordance with the ISO/IEC 17025:2005 Standard for the detection and identification analyses of phytopathogenic fungi and oomycetes included in quarantine lists

- Since January 2014, the Mycology unit had extended is accreditation with a flexible scope to use tests developed and validated in-house.

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Accreditation scope

Standard flexible scope (A2)

- The lower level of flexibility
- No possibility to add new tests without prior evaluation
- If test relies on recognized methods, possibility to implement new versions of this method without prior evaluation in the framework of this level of flexibility

➢ **Used for the detection and morphological identification of phytopathogenic fungi**

Extended flexible scope (B)

- The hightest level of flexibility
- Adding new tests without prior evaluation
- Different sub-levels of flexibility:

  Adoption of test << Adaptation of test<<< Development of test

➢ **Used for the detection of phytopathogenic fungi by PCR developed and characterized in-house**.

Flexible scope of Mycology Unit : general scope

Matrix defined with the AC

Matrice	Organism	Principle of the method
Seeds	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by column absorption. DNA amplification by end (qualitative test)
	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by absorption. DNA amplification by rea (qualitative test)
All parts of plants (except seeds)	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by absorption. DNA amplification by end-poi (qualitative test)
	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acid y column absorption. DNA amplificati by real-time PCR (qualitative test)
Fungi and oomycetes	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by column absorption. DNA amplification by end-point PCR (qualitative test)
	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by column absorption. DNA amplification by real-time PCR (qualitative test)
DNA	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by column absorption. DNA amplification by end-point PCR (qualitative test)
	Phytopathogenic fungi and oomycetes	Manual extraction of nucleic acids by column absorption. DNA amplification by real-time PCR (qualitative test)

For each line, at least 2 tests included

Adding a new matrice, a new organism or a new principle of detection implies an extension of the accreditation (with dedicated evaluation by the AC)

Flexible scope of Mycology Unit : detailed scope

Extract of detailed scope

Matrice	Organism	Principle of the method	Method reference
Seeds of sunflowers	*Plasmopara halstedii*	Detection by grinding, manual extraction, amplification by real-time PCR	MA 032
Leaves, twigs, trunk	*Phytophthora ramorum*	Detection by grinding, manual extraction, amplification by PCR	MOA 018

Why flexible scope ?

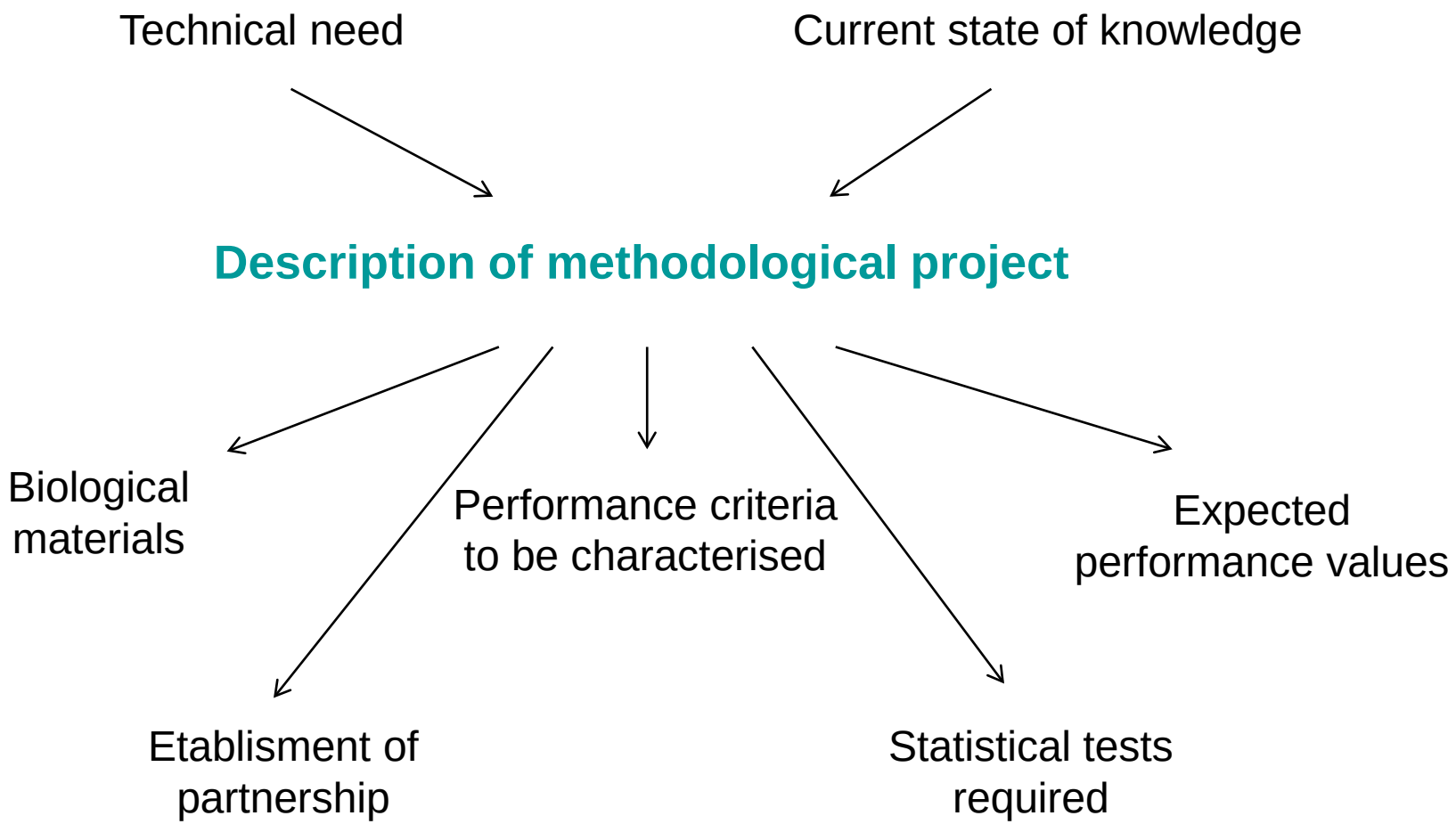
Accreditation under flexible scope enables to:

- Include tests for a quick response to requests (eg phytosanitary crisis situations or emerging parasites)
- Withdraw tests after phytosanitary crisis situations or change in regulations
- Maintain a quality management system that fulfils the requirements of the ISO/IEC 17025 Standard and that is suitable for the unit's size

Evolution of the extent of flexible scope since January 2014

- Inclusion of new tests: **3**
- Revision of tests: **5**
- Withdrawal of tests: **3**

How do you deal with validation ?



How do you deal with validation ?

Criteria to be characterized

For each criteria the process provides:

- A definition
- How it will be assessed
- Expected performance values

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.	

How do you implement quality assurance ?

	Methodological development projects	Analyses under accreditation
Process and traceability	Specific process and forms for the project description and the characterisation of the method	Existing process and traceability for analysis (extraction, PCR, mycological cultures…)
Staff	Definition of a new function: project leader	Existing process and traceability for operators and technical responsibles persons
Management	Specific process for the management of the detailed scope	Existing process and traceability for quality management system
Equipement Consumables Reagents	The laboratory decided to use of the existing quality management system to perform and to trace PCR or real-time PCR detection analyses	

How do you demonstrate expertise

Qualification and maintenance of operator expertise

- Regular activity of detection with molecular biology techniques and specific demonstration when needed (horizontal demonstration, e.g. running PCR on one pest)
- Various controls (positive, negative, specificity, LOD)
- PT or blind sample
- Supervision of work by the project leader during project review

Qualification and maintenance of project leader expertise

- Qualification criteria based on initial training and professional experiences
- Publication in peer-reviewed journals

Noted as a critical point by the quality assessor

Thank you for your attention

More details in Wilson & Ioos (2017) Euroreference 2, 37-45
http://euroreference.mag.anses.fr/sites/default/files/17%2003%20ED%20ER%2002%204_WILSON.pdf