VALITEST webinar series and training activities

What is test validation and why it matters for reliable diagnostics?

2021-01-11

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Introduction of the webinar and training activities

The concept of test validation in Plant Health

Webinar 1	**What is test validation and why it matters for reliable diagnostics?**	**Monday 11th January, 2 pm**
Webinar 2	How to adopt a new test in your laboratory?	Friday 15th January, 2pm
Webinar 3	The use and validation of on-site tests	Wednesday 20th January, 2pm
Practical training session 1	Analysis of performance characteristics	Tuesday 26th of January, 2pm to 4:30 pm
Webinar 4	How do companies handle quality control and validation of products and how will the EPDIA charter help in improving this task?	Monday 1st of February, 2pm
Webinar 5	Why is communication on test selection between risk managers and diagnostic laboratories important ?	Monday 15th of February, 2pm
Practical training session 2	The use of kits: training and demonstration	Thursday 22nd of April, 2pm

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Are you familiar with the concept of test validation?

- **Context of the regulatory plant pest diagnostics and why accurate and reliable tests are needed**

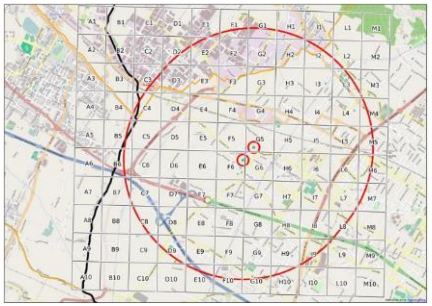
- The choice of a test or a combination of tests depends on the circumstances of use.

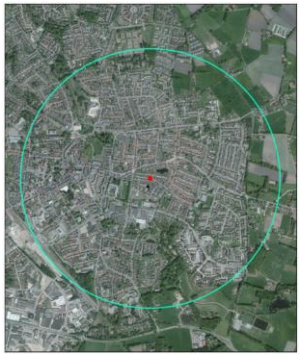
- Validation of tests is part of quality assurance and essential for accreditation

- How is a test validated?

Surveillance to delimit the distribution of known pests and detect new ones

Outbreak management





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Import inspection

Export inspection

- Pre-export crop and consignment inspections
- Issuance of phytosanitary certificates

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Laboratory analysis are needed in the framework of inspection to detect and identify pests

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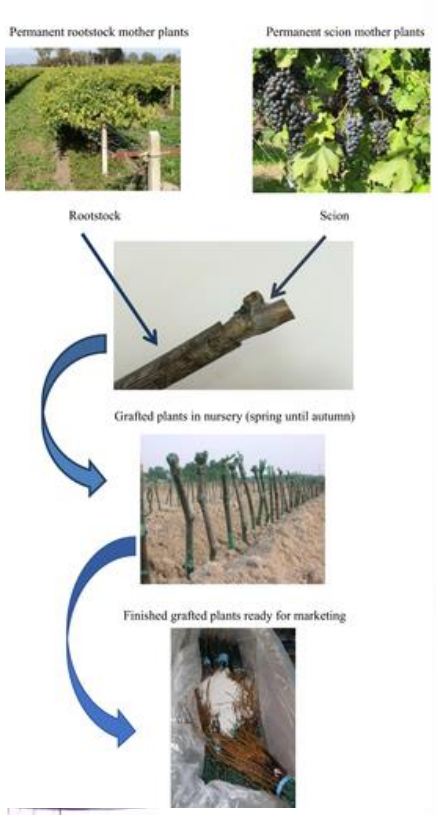
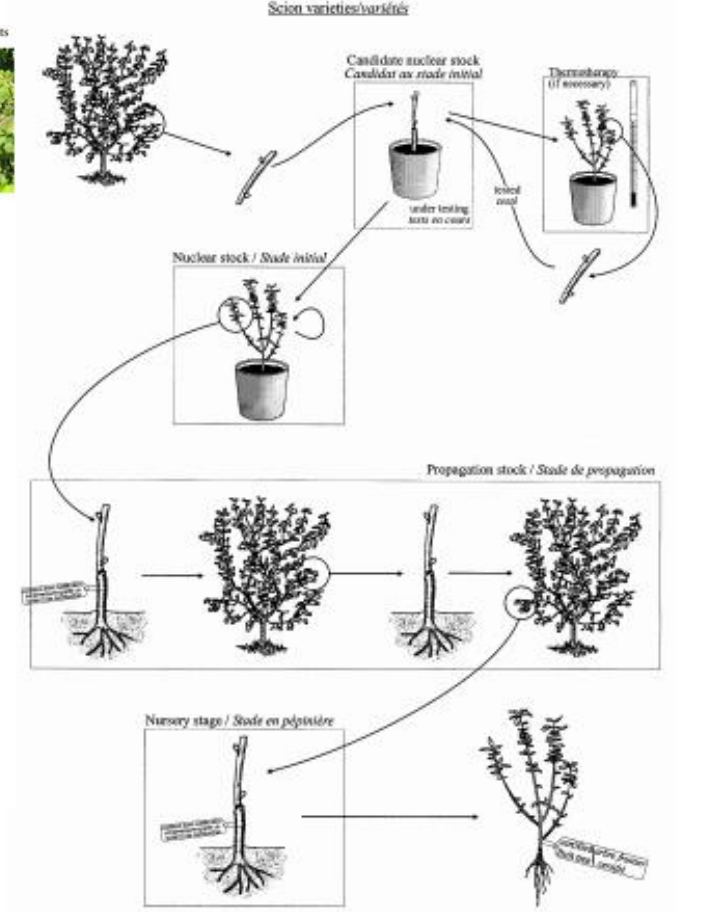
Certification of plant propagating material





Testing is required at different propagation stages to demonstrate the status of the propagating material

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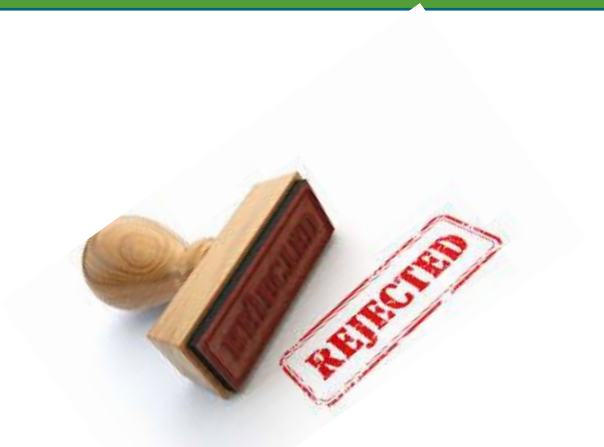
Test(s) results may trigger phytosanitary action (with significant financial, social and environmental consequences)

Eradication of *Xylella fastidiosa* in an almond orchard of the Alicante province (Spain, 2018)
Courtesy: Camille PICARD (EPPO)

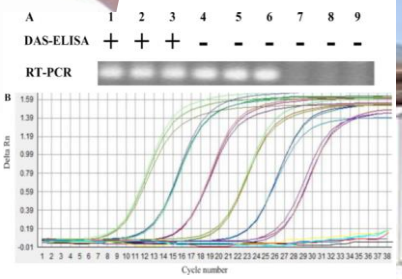
Eradication programmes

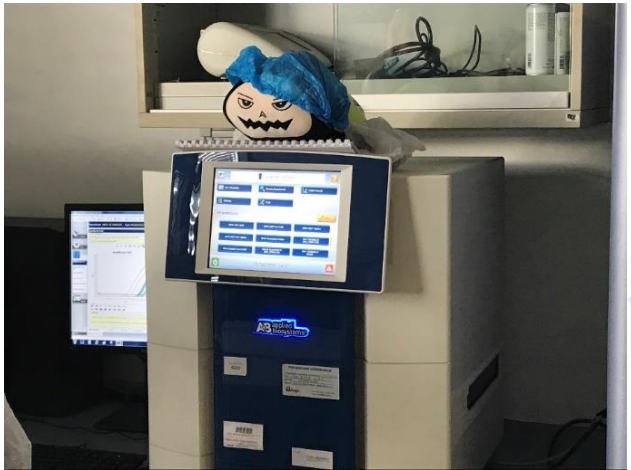
Huanglongbing in citrus groves in Florida.
https://californiacitrusthreat.org/pest-disease/

Consignments being rejected/destroyed at import or export



Rapid detection and accurate diagnosis is essential





- Context of the regulatory plant pest diagnostics and why accurate and reliable tests are needed

- **The choice of a test or a combination of tests depends on the circumstances of use.**

- Validation of tests is part of quality assurance and essential for accreditation

- How is a test validated?

Diagnostic tests are used in different circumstances

- Routine survey(s) for the diagnosis of a pest widely established in a country
- General surveillance for pest status (e.g. demonstrate freedom)
- Surveillance for latent infestations by pests
- Surveillance as part of an official control or eradication programme
- Pest diagnostic associated with phytosanitary certification or certification of propagation material
- Routine diagnosis for pests in imported consignments
- Detection of a pest in an area where it is not known to occur
- Detection of a pest in a consignment originating in a country where the pest is declared to be absent.

Depending on the circumstances, different test characteristics are needed

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First let's understand each other

Assay

Method

Protocol

Test

Diagnostic Protocol

Different terms are used and may not have the same meaning

At EPPO the decision was made to use the following terminology:

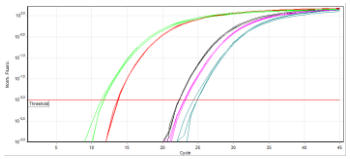
EPPO Diagnostic Protocol

=

TEST = Method (e.g. real-time PCR) + Target pest (ToBRFV) + Matrix (tomato seeds)

Detection

Identification



ToBRFV?

Combination of tests

- Context of the regulatory plant pest diagnostics and why accurate and reliable tests are needed
- Tests should be chosen depending on their circumstances of use.
- **Validation of tests part of quality assurance and essential for accreditation**
- How is a test validated?

Quality assurance & accreditation

It is essential for laboratories to work under quality assurance and

validation of tests is done under quality assurance

Quality systems consist of activities that ensure the quality of and confidence in the results provided by a laboratory.

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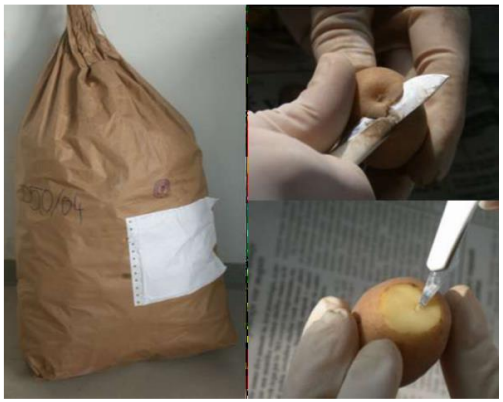
Who in the audience works in a laboratory with a quality assurance system?

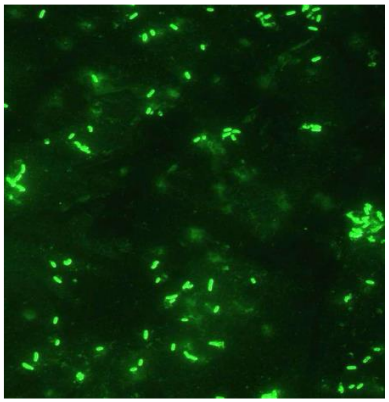
Minimum general quality management requirements needed to perform diagnostic tests for plant pests in a laboratory have been agreed in the EPPO region

PM 7/84

Standard on basic requirements for quality assurance

first approved in 2007 revised in 2018





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Quality management: management system

Facilities and activities of the laboratory e.g.

- resources are available to conduct the plant pest diagnostic activity (e.g. personnel, facilities and consumables)
- responsibilities and tasks of personnel are clearly defined
- procedures and instructions are available e.g. standard operating procedures (SOPs)
- confidentiality and conflicts of interests……..
- internal audits
- documentation

Quality management: technical requirements

Factors which determine the reliability of the test results.

- Qualified personnel
- Appropriate facilities and environmental conditions
- Selection of plant pest diagnostic tests
- Equipment
- Reference materials/cultures
- Sampling
- Sample handling
- Controls
- Reporting results

Accreditation

Accreditation of laboratories in the EPPO region is according to ISO/IEC 17025
(*for some labs it is a legislative requirement*)
To achieve harmonization of interpretation of the ISO Standard for plant pest diagnostic laboratories an EPPO Standard was approved

PM 7/98

2009

Standard on the interpretation of ISO 17025 for plant pest diagnosis

includes guidance per discipline on how to perform the validation of a test

Revisions approved in 2014, 2018 & 2019

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Does your laboratory perform tests under ISO 17025 accreditation?

- Context of the regulatory plant pest diagnostics and why accurate and reliable tests are needed
- Tests should be chosen depending on their circumstances of use.
- Validation part of quality assurance and essential for accreditation
- **How is a test validated?**

A laboratory preparing for accreditation should only use validated tests

Validation of a test =
Evaluation of its performance characteristics

Different performance criteria used to characterize tests:

Analytical sensitivity (how much)
Analytical specificity (what)
Reproducibility (effect of operator, time of analysis, equipment)
Repeatability (consistent results between replicates)
Depending on the scope of the test selectivity (matrix variation) may also need to be determined.

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Just a quick slide to explain the difference between **validation** and **verification**

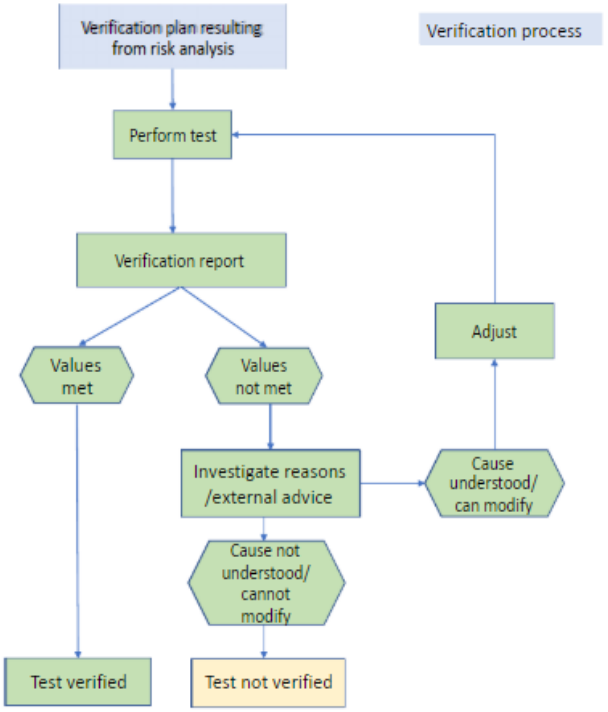
Laboratory performing a test

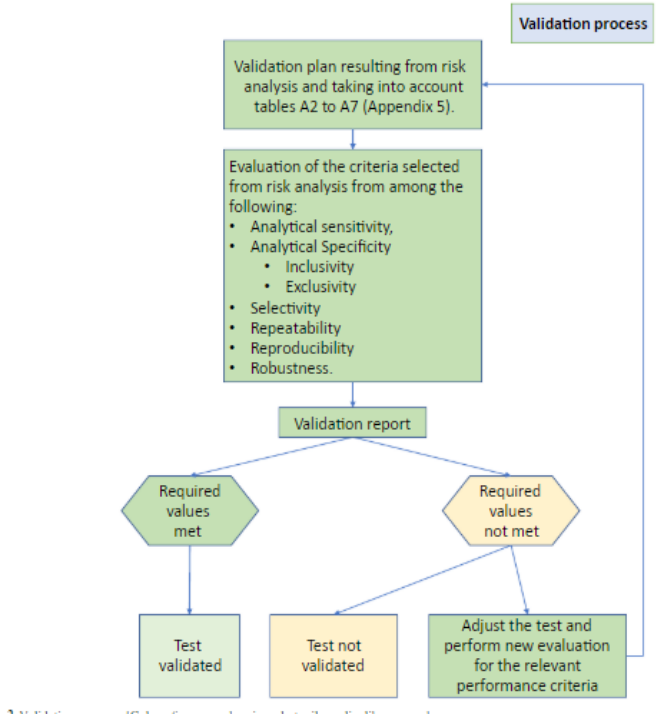
Test with validation data

Laboratory performs a **verification**
(to confirm its competence in performing the test).

Test with no or incomplete validation data

Laboratory should produce the missing **validation** data





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Let's look a little bit more in what these criteria are

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Analytical sensitivity

Smallest amount of target that can be detected reliably (this is sometimes referred to as 'limit of detection').
Further details on the procedures to determine analytical sensitivity are given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*

Analytical specificity

Inclusivity: The performance of a test with a range of target organisms covering genetic diversity, different geographical origin and hosts

Exclusivity: Performance of a test with regards to cross-reaction with a range of non-targets (e.g. closely related organisms, contaminants)

Repeatability

The level of agreement between replicates of a sample tested under the same conditions

Reproducibility

The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, persons, equipment, location)

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Selectivity

The extent to which variations in the matrix affect the test performance (matrix effect)

Diagnostic sensitivity

Proportion of infected/infested samples testing positive compared with results from an alternative test (or combination of tests). Sensitivity = true positives/(true positives + false negatives)

Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from an alternative test (or combination of tests). Specificity = true negatives/(true negatives + false positives)

Diagnostic specificity

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A laboratory preparing for accreditation should only use validated tests

Validation of a test =
Evaluation of its performance characteristics

Different performance criteria used to characterize tests:

Analytical sensitivity (how much)
Analytical specificity (what)
Reproducibility (effect of operator, time of analysis, equipment)
Repeatability (consistent results between replicates)
Depending on the scope of the test selectivity (matrix variation) may also need to be determined.

First step : consider which performance criteria need to be evaluated based on a risk analysis

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RISK ANALYSIS

Scope:
organism, matrix, method

Context of use

input

Risk analysis to identify which performance criteria need to be evaluated and to what extent

Intended use of the test screening, on-site testing, confirmation test…
Specific needs of the client?
Impact of the result
Level of confidence needed?
For pests with large host range, priority hosts
Specific species strains to be detected?
Evaluation needs for possible cross reactions?
Flexibility needed?

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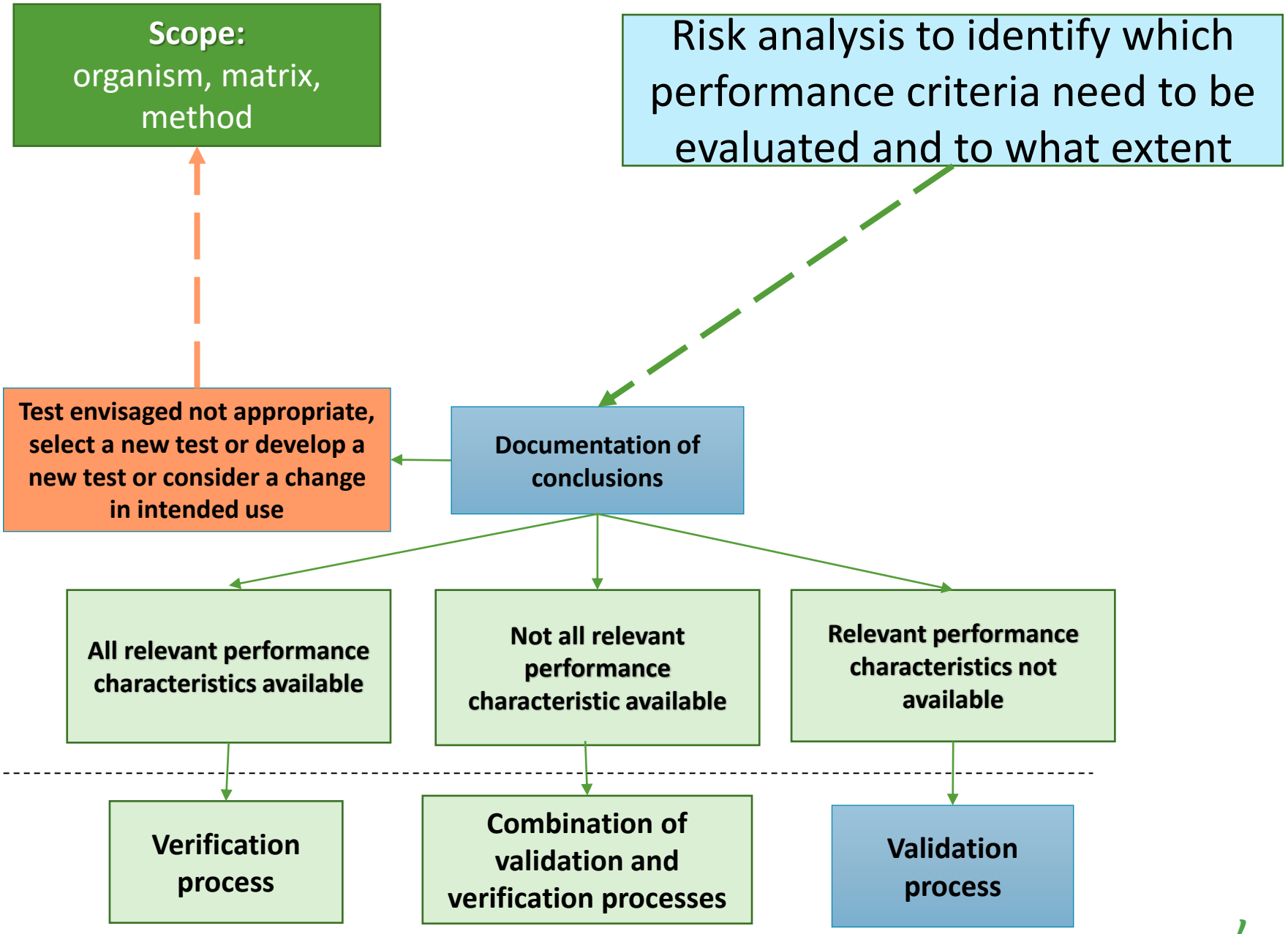
Scope:
organism, matrix, method
Risk analysis to identify which performance criteria need to be evaluated and to what extent
Test envisaged not appropriate, select a new test or develop a new test or consider a change in intended use
Documentation of conclusions
All relevant performance characteristics available
Not all relevant performance characteristic available
Relevant performance characteristics not available
Verification process
Combination of validation and verification processes
Validation process

Examples of outputs of the risk analysis

Transferable skills:
If the data from a test using the same method is transferable from a test for another pest (e.g. matrices are comparable), this could mean that the extent of validation can be reduced (e.g. for selectivity, repeatability, reproducibility).
Experience with real-time PCR for Flavescence dorée would allow the extent of validation for repeatability and reproducibility for Bois Noir to be reduced.

Analytical sensitivity:
If the quantity of target in the sample is not a limiting factor, the extent of validation for analytical sensitivity can be reduced.
Example: Identification on pure culture by PCR, as long as there is no inhibition effect (excess of matrix).

Analytical specificity: If the test cannot distinguish between genera or species within a genus, then inclusivity and exclusivity evaluations can be reduced. Example: Nematode extraction methods are not specific for one species or one genus.

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How to generate performance characteristics?

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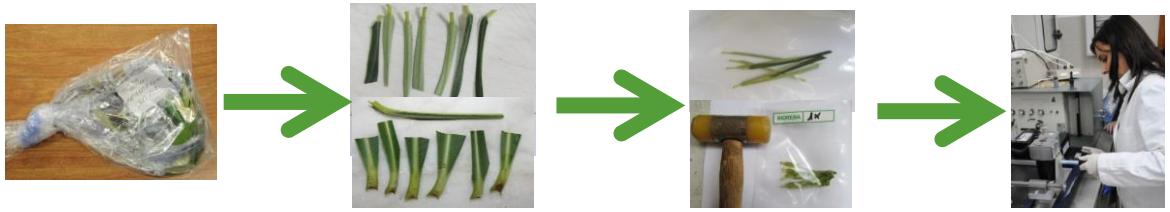
The EPPO Standard PM 7/98 includes annexes providing recommendations for the evaluation of performance criteria per discipline and for different methods

Example in bacteriology for molecular methods

Molecular methods, e.g. PCR, real-time PCR, LAMP This step also includes methods for the isolation of DNA from the matrix.	
Analytical sensitivity	Analyse at least three series of spiked sample extracts with a range of 10^1–10^6 cells of the target organism per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result. If consistent results are not obtained after three series, then additional series should be prepared and tested.
Analytical specificity	Inclusivity: analyse strains of the target bacterium covering genetic diversity, different geographic origin and hosts. Exclusivity: analyse a set of non-target bacteria, in particular those associated with the matrix. For both inclusivity and exclusivity use cell suspensions of pure cultures at approximately 10^6 cells per mL and use antiserum/antibodies at their working dilution.
Selectivity	Determine whether variations in the matrix (e.g. by using different hosts of the same family, different cultivars of the host plant) affect the test performance.
Repeatability	Analyse at least three replicates of spiked sample extracts with a low concentration. If consistent results are not obtained, additional replicates should be prepared and tested.
Reproducibility	As for repeatability, but with different operator(s) if possible, on different days and with different equipment when relevant.



Analytical sensitivity

Molecular methods, e.g. PCR, real-time PCR, LAMP

Perform at least three experiments with spiked sample extracts with a range of 10–10^6 cells of the target bacterium per mL. Preferentially, this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result. If consistent results are not obtained after three experiments, then additional experiments should be conducted.

Analytical specificity

Molecular methods, e.g. PCR, real-time PCR, LAMP

Inclusivity: analyse a range of strains of the target bacterium covering genetic diversity, different geographic origin and hosts

Exclusivity: analyse a set of non-target bacteria, in particular those associated with the matrix.

Use cell suspensions of pure cultures at approximately 10^6 cells per mL. For non-targets, the NA concentration should be high enough to maximize the possibility of cross reaction but remain realistic.

For both inclusivity and exclusivity, the test results can be supported by 'in silico' comparison of probe/primer sequences to sequences in genomic libraries.

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Repeatability

Molecular methods, e.g. PCR, real-time PCR, LAMP

Analyse at least three replicates of spiked sample extracts with a low concentration determined by the results from the analytical sensitivity experiments. If consistent results are not obtained, additional replicates should be prepared and tested.

Reproducibility

Molecular methods, e.g. PCR, real-time PCR, LAMP

As for repeatability, but with different operator(s) if possible, on different days, and with different equipment when relevant.

Selectivity

Molecular methods, e.g. PCR, real-time PCR, LAMP

Determine whether variations in the matrix (e.g. by using different age/conditions of plant material or different cultivars of the host plant) affect the test performance

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Database on diagnostic expertise major update in 2020 done in the framework of Valitest

EPPO database
on Diagnostic Expertise

HOME Laboratory List Expertise List Technical A

Encourage collaboration and communication between laboratories

Country		Quality Prog
Austria	AGES Institute of Sustainable Plant Production Vienna	YES
Austria	Department of Forest Protection Wien	YES
Azerbaijan	Central phytosanitary laboratory of the Azerbaijan Food Safety Institute Baku	YES
Belarus	department of quarantine expertise Minsk	YES

EPPO database
on Diagnostic Expertise

HOME Laboratory List Expertise List Technical Auditors/Experts list Validation data for diagnostic tests Connect to my Lab

Validation reports generated by laboratories or in the framework of projects available through the database

Organism(s)	Matrix(ces)	Plant species tested	Detection / Identification	Methods		Follow	Follow	Data	Report	LabID	PDF
				action, Morphological		yes		no	2008-12-31	ILVODGB	
Xylella fastidiosa	Herbaceous cuttings	Brassica oleracea var. capitata, Vinca minor	detection	Extraction, Molecular Extraction DNA RNA, Molecular real time PCR	no, yes	no, yes	no	no	2019-03-13	ISPP-CNR	

EPPO database on Diagnostic Expertise

HOME | Laboratory List | Expertise List | Technical Auditors/Experts list | Validation data for diagnostic tests | Connect to my Lab

LIST OF VALIDATION DATA

38 validation data displayed

Organism(s)	Matrix(ces)	Plant species tested	Detection / Identification	Methods	Kit	Follow EPPO diag	Follow IPPC diag	Data referred to EPPO	Report date	LabID	PDF
Erwinia amylovora	Leaves, Shoots	Rosaceae	detection	Extraction, Isolation		yes	no	yes	2012-03-01	IVIA-B	
'Candidatus Phytoplasma mali', 'Candidatus Phytoplasma prunorum', 'Candidatus Phytoplasma pyri'	Leaves, Roots, Specimen, Wood, Woody cuttings	Malus domestica, Prunus sp., Pyrus communis	detection	Molecular Extraction DNA RNA, Molecular real time PCR	no	no	no	yes	2014-05-28	NIB-FITO	
Pantoea stewartii subsp. stewartii	Other	Zea mays	detection and identification	Serological IF	yes	yes		yes	2011-05-31	EUPH-PANTOEA	
Xylella fastidiosa	Herbaceous cuttings	Brassica oleracea var. capitata, Vinca minor	detection	Extraction, Molecular Extraction DNA RNA, Molecular	no, yes	no, yes	no	no	2019-03-13	ISPP-CNR	

How to use the validation data to decide on the test to use some examples

Practical training session 1	Analysis of performance characteristics	Tuesday 26th of January, 2pm to 4:30 pm (Registration closed)

Xylella fastidiosa: not an easy pest!

Sub-species

- *fastidiosa*
- *pauca*
- *multiplex*
- sandyi…

Vectors

- Cercopoidea (spittlebugs and froghoppers)
- Cicadoidea (cicadas)
- Membracoidea (Cicadellidae: Cicadellini)

More than 300 hosts

- Olive
- Vitis
- Prunus
- Coffee
- Citrus
- *Polygala myrtifolia*
- *Nerium oleander*
- Lavender
- ……

Validation of tests is a challenge!

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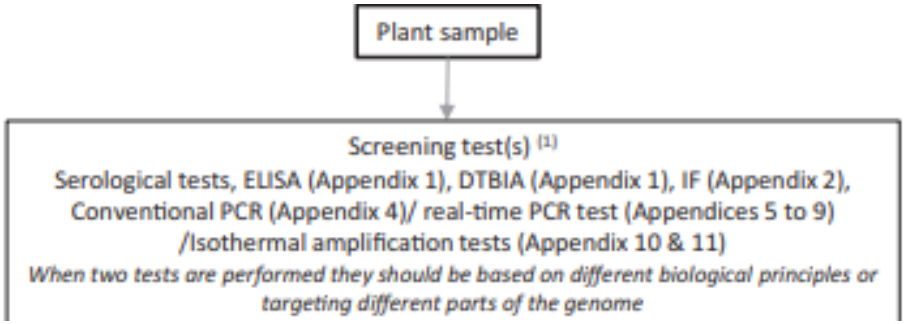
Xylella fastidiosa (EPPO diagnostic protocol PM 7/024)



More sensitive tests (lower analytical sensitivity) needed to ensure a first detection in areas where pests not known.

Lack of analytical specificity can be accepted for screening tests as two tests needed to confirm a detection

A molecular test(s) should be performed for the detection on **asymptomatic plant material from a pest free-area**
For testing of **symptomatic plants** from a known outbreak area or a buffer zone around an outbreak a single test including serological tests (e.g. ELISA) may be considered sufficient.

Analytical sensitivity may vary depending on the matrix.
Nucleic acid extraction is critical and different NA extraction methods may result in different analytical sensitivity

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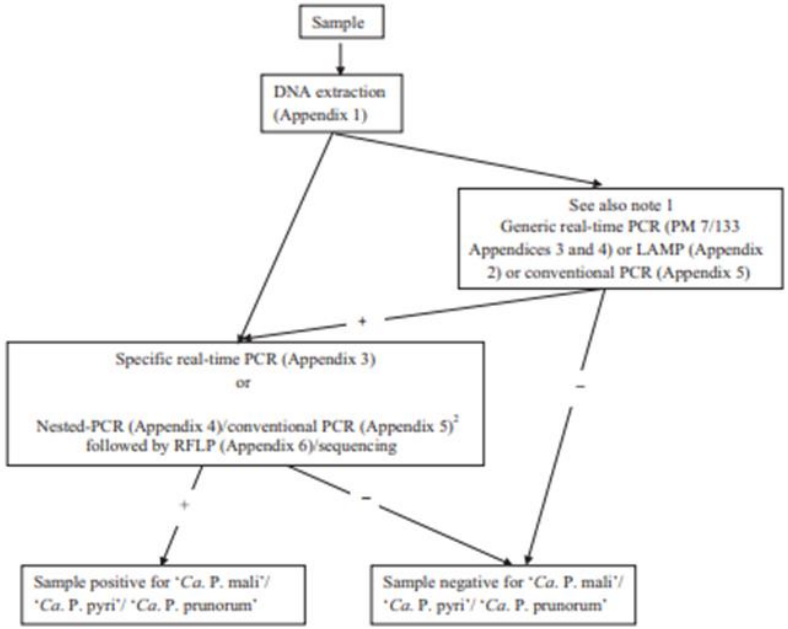
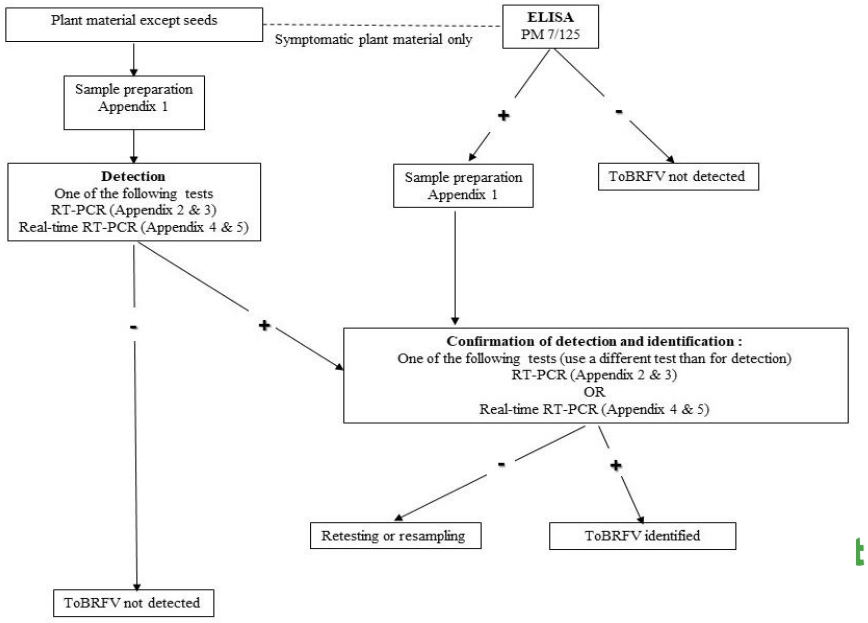
‘*Candidatus* Phytoplasma mali’, ‘*Ca*. P. pyri’ and ‘*Ca*. P. prunorum’ (EPPO diagnostic protocol PM 7/062)



Depending on the circumstances of use (e.g. imported plant material versus plant material tested for a specific phytoplasma survey) it may be useful to perform a generic test which would then detect other phytoplasmas.

Tomato brown rugose fruit virus (new diagnostic protocol)



ELISA cross reaction noted and analytical sensitivity level not considered sufficient

Some PCR tests not included because of cross reaction as well.

And many more examples

- *Clavibacter insidiosus* tests recommended on pure cultures but not on plant/seeds due to lack of data on plant/seed extracts or poor analytical sensitivity.

- *Phyllosticta citricarpa:* lack of specificity of current tests to distinguish *P. citricarpa* and *P. paracitricarpa.* Need for culturing and sequencing.

- *Erwinia amylovora: Other bacteria can cause fire blight-like symptoms* e.g. *Erwinia pyrifoliae, Erwinia piriflorinigrans*

Need for communication between diagnosticians and risk managers on test selection

Webinar 5	Why is communication on test selection between risk managers and diagnostic laboratories important ?	Monday 15th of February, 2pm

Thank you for your attention!

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