

Analysis of performance characteristics

2021-01-26

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139



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?	11 th January
Webinar 2	How to adopt a new test in your laboratory?	15 th January
Webinar 3	The use and validation of on-site tests	20 th January
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 1 st of February, 2pm
Webinar 5	Why is communication on test selection between risk managers and diagnostic laboratories important ?	Monday 15 th of February, 2pm
Practical training session 2	The use of kits: training and demonstration	Thursday 22 nd of April, 2pm

Agenda:

- **Welcome**
- **Theory behind performance characteristics**
- **Discussion in smaller groups**

Coffee-tea-break

- **Findings and conclusions**
- **Wrap up**

Poll:
Do you have experience
with validated assay's in
your lab?

Yes: raise hand

- **Recap: Concept of validation**
- Performance characteristics
- Examples/cases



There are many reasons for testing your crops:

- Detect new plant diseases and/or pests
- Monitor the distribution of known diseases and pests

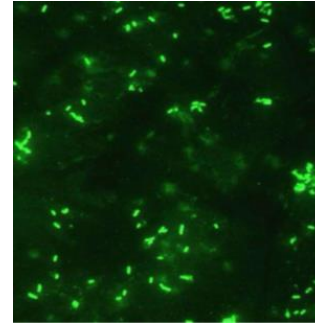


Rapid, accurate and reliable detection methods are important!

- Phytosanitary consequences:
 - Use of pesticides against pathogens or vectors
 - Other measures to control spread
 - Phytosanitary measures, eradication

Which test to use and why:

- Depending on the needs:
 - Routine testing
 - Testing for diagnosis
 - Certification
 - Find emerging pathogens/pests
 - Surveillance



Different circumstances => different needs => different performances

It is essential for laboratories to work under quality assurance and

validation of tests is done under quality assurance

Factors that influence the reliability of a test:

- **Facilities: personnel, equipment, lab conditions**
- **Materials: controls, references, sample material**
- **Methods: selection of diagnostic test, sampling, sample preparation**

Harmonisation is important!

Quality system

Quality system: PM 7/84 as a standard on basic requirements for quality assurance

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

Accreditation

To achieve harmonization of interpretation of the ISO Standard for plant pest diagnostic laboratories an EPPO Standard was approved:

PM7/98

How to interpret ISO 17025
Guidance per target species

Validation: reliable diagnostic tests, quality assurance

Accreditation: to achieve harmonisation between
diagnostic laboratories

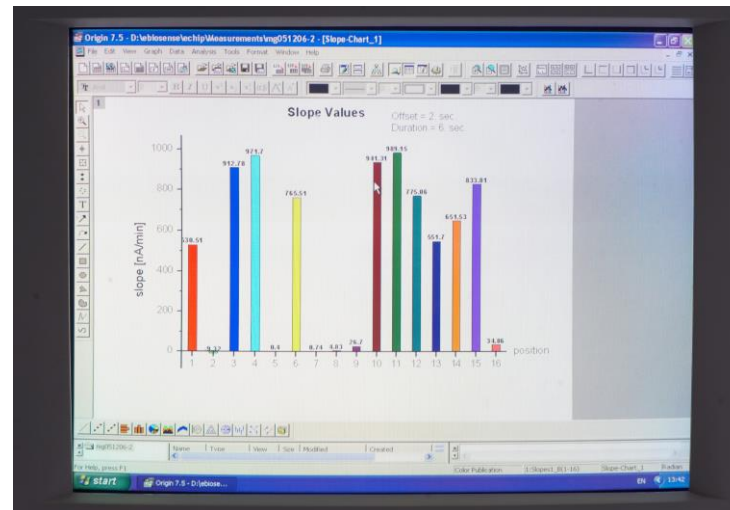
**Validation of a test is the evaluation of its performance
characteristics**



Poll:
Do you work with assay's
under ISO17025
accreditation?

Yes: raise hand

- Recap: Concept of validation
- **Performance characteristics**
- Examples/cases



Performance characteristics and criteria



Performance criteria:

Variable which is measured in a given test

Different performance criteria are used to characterize a test

Performance characteristics:

the values of performance criteria for a given specific test

Performance criteria

Tests are characterized by their performance criteria:

Analytical sensitivity (**how much**)

Analytical specificity (**what**)

Reproducibility (**effect of operator, time of analysis, equipment**)

Repeatability (**consistent results between replicates**)

Selectivity (**matrix variation**)

Robustness (**sensitivity for deviations**)

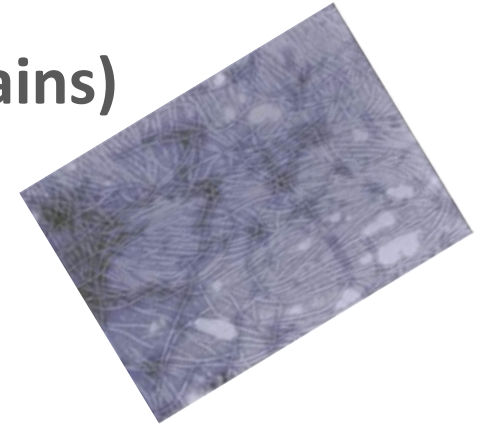
Analytical Sensitivity:

- Smallest amount of target that reliably can be detected (limit of detection)
- How: analyze a dilution series of samples, or artificial subsamples created from one sample, until limit of detection is reached
- Challenge: how to determine the exact amount of target when a pathogen:
 - cannot be cultured (obligate pathogens)
 - cannot be quantified (fungi)
 - cannot be purified (e.g. phytoplasmas)



Analytical Specificity:

- Does a test detect only the specific target?
- How: test a panel of related targets (e.g. different strains) and non-targets available at medium concentrations
- Challenge: collections available
- Inclusivity: analyse a set of strains of the target organism covering genetic diversity, different hosts and geographic origin
- Exclusivity: analyse a set of non target but closely related organisms, especially the ones associated with the matrix. Look for cross-reactivity with the organisms and/or the possible contaminants



Repeatability:

- Are the results of a test consistent between replicates?
- How: analyse at least three replicates of the same sample at a low concentration of target
- If no consistent results are obtained; additional replicates should be prepared and tested.

Reproducibility:

- What is the effect of external factors on a test? External factors being: persons, equipment, time, place
- How: like repeatability but at different moments, when possible with different operators and different equipment

Selectivity:

- Effect of the matrix on the test?
- How: analyse at least three replicates of a specific matrix relevant for the organism
- Challenge: availability of matrices

Robustness:

- Effect of deviations in protocol or performance
- How: included in the reproducibility
- Separate evaluation is often not necessary



Diagnostic vs analytical

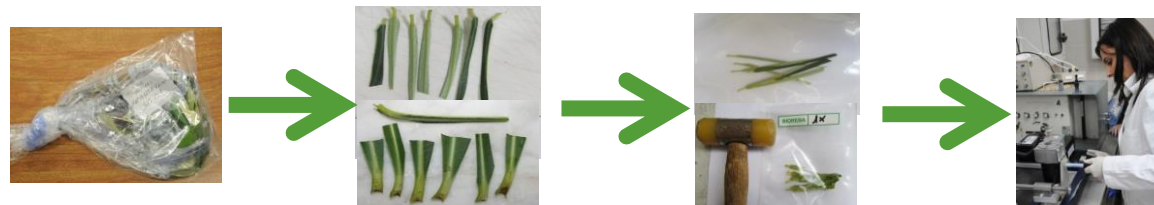
- **Analytical sensitivity:**
 - The smallest amount of target a test can detect
 - Limit of detection of a test
- **Diagnostic sensitivity:**
 - Proportion of infected samples being tested positive compared with results from alternative (combination of) tests
 - $\text{True positives} / (\text{true positives} + \text{false negatives})$
- **Analytical specificity:**
 - The ability of a test to detect correctly the target
- **Diagnostic specificity:**
 - Proportion of healthy samples being tested negative compared with results from alternative (combination of) tests
 - $\text{True negatives} / (\text{true negatives} + \text{false positives})$

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



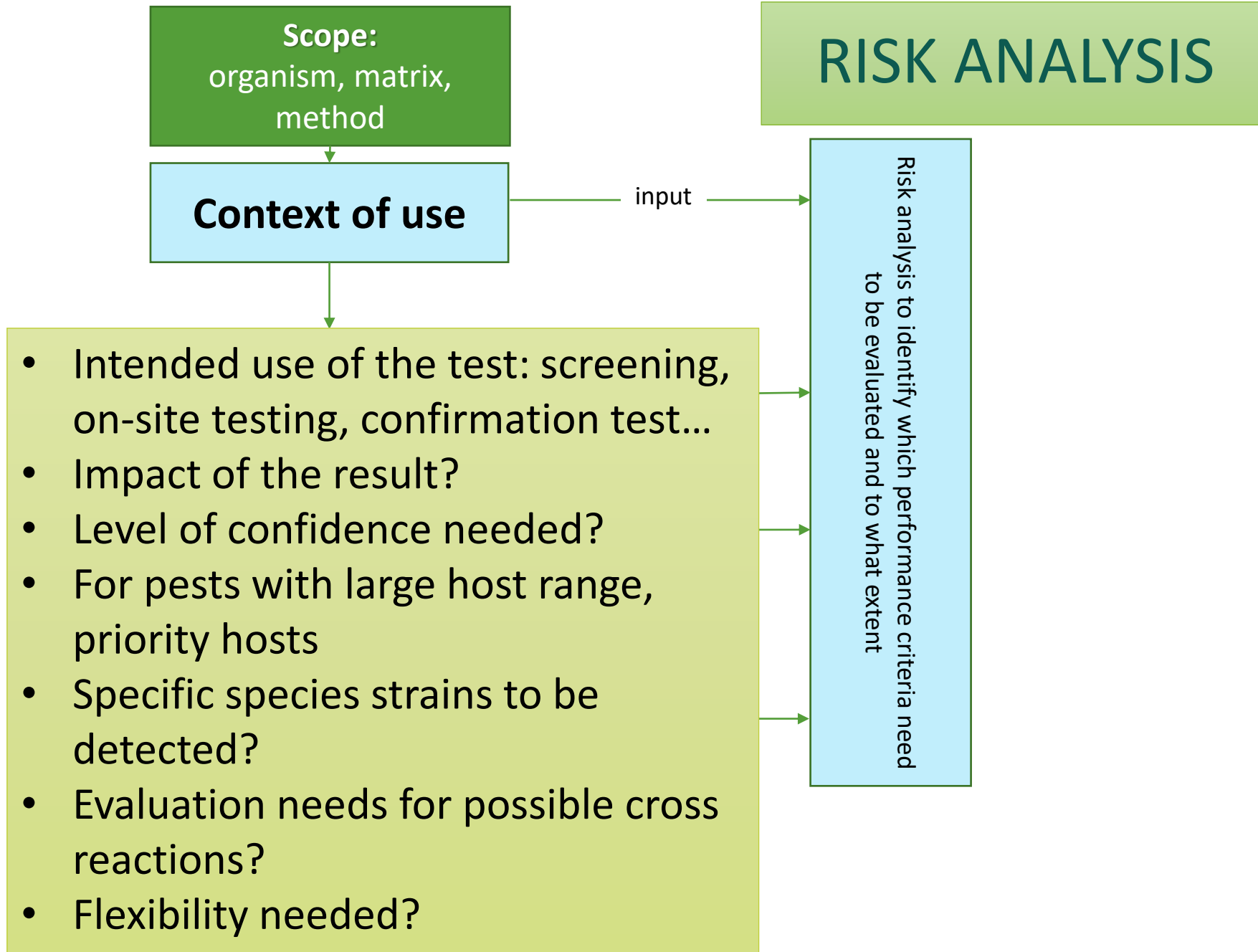
Example in bacteriology for molecular methods

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.



- Recap: Concept of validation
- Performance characteristics
- **Examples/cases**

This is an example !



Which performance criteria to evaluate?

1. Make a risk analysis
2. Determine which criteria need to be evaluated and to what extent



Scope: organism, matrix and method
Intended use, constraints, availability of materials



Questions:
Impact of the result, needs, flexibility, priorities



An example: *Xylella fastidiosa*

Quarantine organism (EPPO A2 list)

300 hosts, 3 vectors, 4 subspecies

You want to 'find emerging pathogens'

Which performance criteria would you enter in your risk analysis?

Answer in the chat or write it down for yourself

An example: *Xylella fastidiosa*

**Different tests available: molecular and serological
but....**

Analytical sensitivity and analytical specificity can be a problem

SO....

Diagnostic protocol PM 7/024

Matrix!

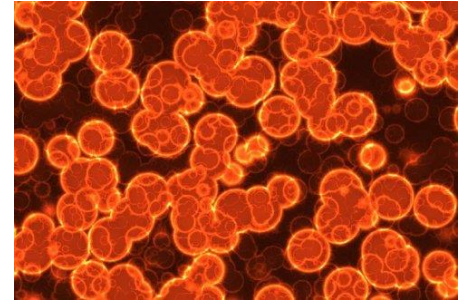
Asymptomatic material from a pest-free area!

Perform 2 different tests for confirmation



Some more examples

- *Erwinia amylovora*
 - Challenge: other bacteria can cause fire blight-like symptoms as well e.g. *Erwinia pyrifoliae* and *Erwinia piriflorinigrans*
- *Tobamoviruses*
 - Challenge: cross-reactivities in ELISA
- *Petunia*
 - Challenge: 14 targets to test



Thank you for your attention!

質問は？

Vragen zijn welkom

Irgendwelche Fragen?

Questions?

Avez-vous des questions?

¿alguna pregunta?

любые вопросы?

Qualsiasi domanda?

frågor?



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