

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 11 th January, 2 pm
Webinar 2	How to adopt a new test in your laboratory?	Friday 15 th 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 26 th 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

VALITEST webinar series and training activities

3 - webinar VALITEST

The use and validation of on-site tests

20.01.2021



Denise Altenbach (Manufacturer view)



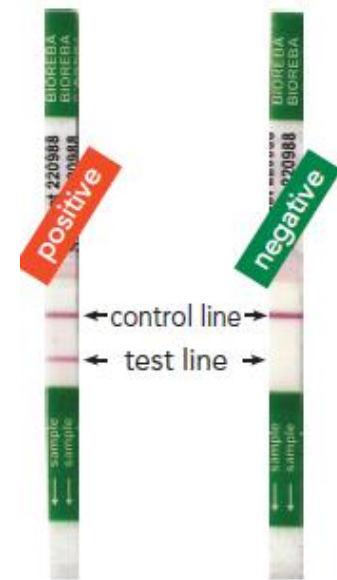
Jean-Philippe Renvoisé (User view)



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



What are on-site tests?

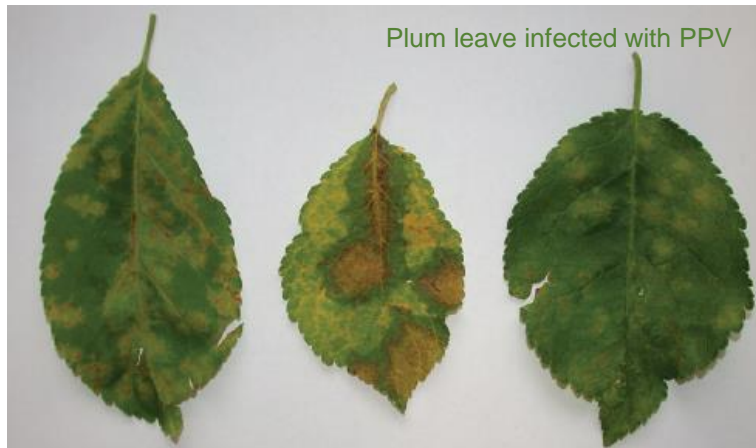


- fast assay, results within 15 minutes
- optimal for single testing
- easy handling
- suitable when testing on-site in greenhouse or field
- no need for special laboratory equipment

Note: there are on-site tests based on molecular methods (isothermal amplification).

What do you need to perform an on-site test?

Symptomatic tissue



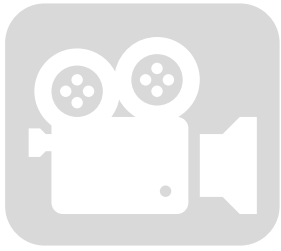
Trained staff



AgriStrip Complete Kit



Trained for:
sampling & performing the test



Video

Example of Agristrip workflow

When / how to use on-site tests?

Needs Context of use

- type of matrix ? leaves ?
- symptomatic or not ?
- test season: are there constraints?



Performance characteristics Intended use

Do the needs fit with the
«intended use» of an on-site test?

- Information can be found on the manufacturers Product Information
- Identify modifications ?
- Additional validation required due to modifications ? Verification sufficient ?
- Is there a need to train the test user ?
- Please ask your supplier for advice! We know our tests!

Product Information PPV AgriStrip

Product Information: AgriStrip Plum pox virus (PPV; Sharka)

PPV AgriStrip - a rapid assay for the detection of Plum pox virus (PPV)

Intended use

The rapid assay PPV AgriStrip is produced by BIOREBA for identification of PPV (Sharka) in *Prunus* sp. showing symptoms such as chlorotic spots, blotches, bands, rings or line patterns on leaves (Fig. 3,4). Later symptoms include uneven ripening, blotching and rings on fruits (Fig. 1). The concentration of PPV in tissues of fruit trees may vary considerably. For example, in peach and apricot trees, the concentration varies even within the same leaf.

Fig. 1. Sharka symptoms on fruit



These antibodies have been truly validated with extensive collections of virus isolates from over 20 different countries; e.g. at Palacky University in Olomouc as well as in independent studies „ringtests“), carried out at IVIA, Valencia, Spain (COST 88 PPV workshop) and at the Virological Laboratory Gödöllő, Hungary, where all isolates from different host and geographic origins were detected.

The sensitivity attained with these antibodies in the AgriStrip lateral flow is very similar to the sensitivity in the DAS-ELISA format (less than 2-fold difference in dilution series up to 1:640, w/v). In parallel tests with field samples of apricot, peach and plum, the accordance between the two test formats was 100% so far.





Poll

Have you already used an on-site test?

Have you already tried to validate an on-site test?

The use and validation of on-site tests

(Kit manufacturer view)

Denise Altenbach



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Manufacturer view: Development and Validation: Example PPV AgriStrip

- **Selection of antibodies**
- Test antibody functionality in LFD format
- Validation: Specificity / Sensitivity / Sampling optimization
- Definition of the “Scope: Intended Use”
- Production and standardization: lot-to-lot consistency
- Customer training

Pre-selection of antibodies: for development and validation of the PPV AgriStrip

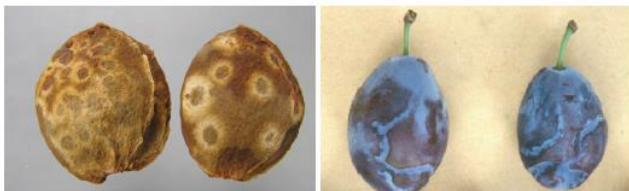
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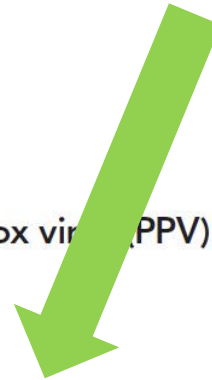
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Fig. 1. Sharka symptoms on fruit



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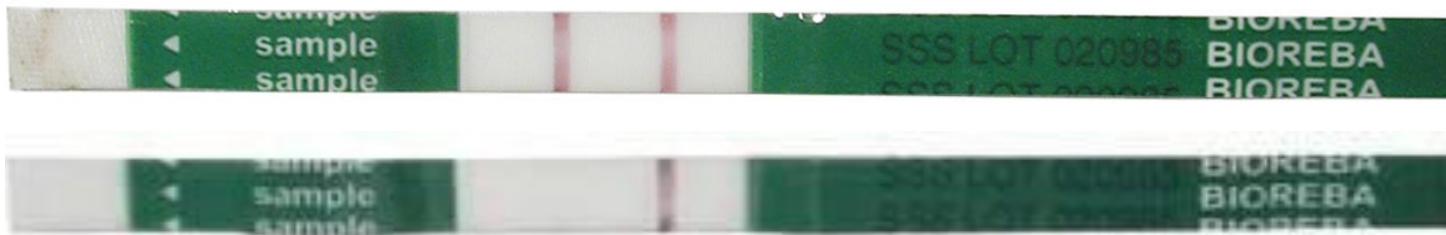
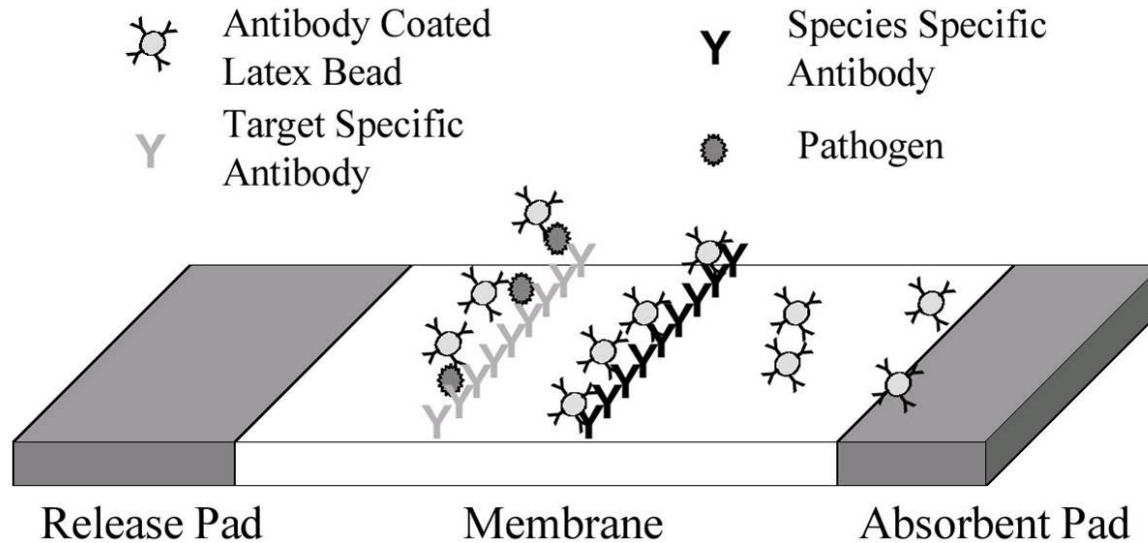
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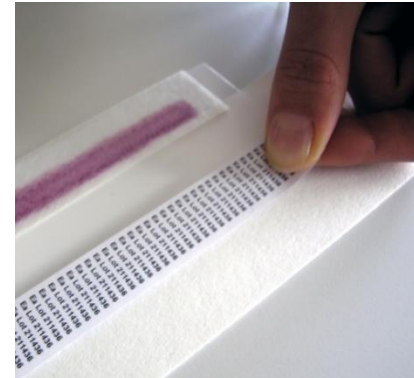
- Selection of antibodies
- **Test antibody functionality in LFD format**
- Validation: Specificity / Sensitivity / Sampling optimization
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Check suitability of antibodies in LFD format (application on membrane, coupling to colloidal gold particles)



This is how a production is done

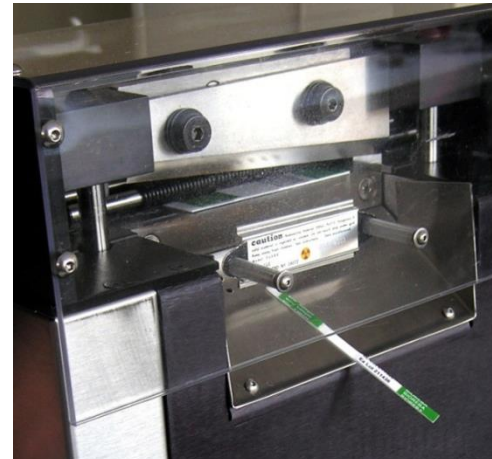
Coating antibodies to the membrane



Coupling antibodies to gold particles



Gold is sprayed on glass fiber
Assembly of the card components



Cutting the card in strips

Manufacturer view: Development and Validation: Example PPV AgriStrip

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Validation: Sensitivity / Specificity / Sampling optimization

Validation & protocol optimization go “hand in hand”



Main criteria are: **Analytical sensitivity / Analytical specificity**



Once this is “satisfactory”, **other criteria** are taken into account:

- **Repeatability**
- **Reproducibility**
- **Stability**
- **Lot-to-lot consistency ...**

Validation criteria: PPV AgriStrip

Analytical specificity (inclusivity / exclusivity):

- Detection of **all PPV isolates**
- No **cross-reactivity** with other viruses occurring on the same host

Selectivity

- **Selectivity:** Low/no **background** with healthy host tissue (leaves, petals etc. of *Prunus* sp.)

Analytical sensitivity not at the cost of selectivity

- Low **analytical sensitivity** versus low **background** with healthy samples
- Lateral Flow: Dilution of the **matrix** (secondary plant compounds with neg. effect on test performance)

Playground for optimization

Playground
for optimization



Factors with impact on
sensitivity and specificity

Formulation

- **antibody mix**: which antibodies are coupled to colloidal gold and/or to the membrane
- **concentration** and ratios of applied antibodies

BIOREBAs job

Sample preparation

- sample extraction **buffer**
- sample **dilution**
- **extraction** procedure
- use of recommended sample **tissue**

Joint job: User &
Manufacturer



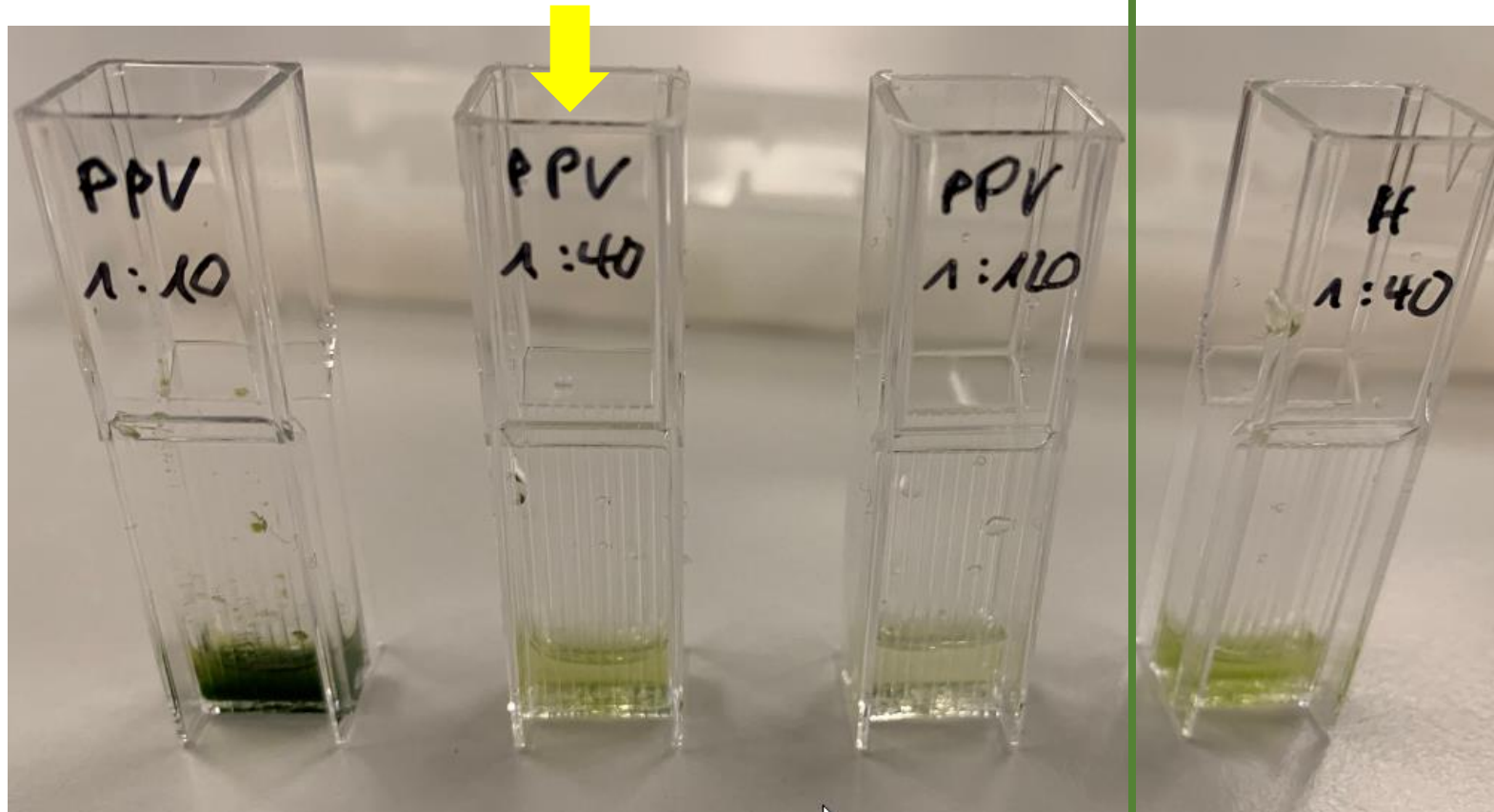
Optimized sample preparation

Movie 2: Optimized extraction buffer & performance of extraction

Sample size and sample dilution (w/v) optimized

Summary: Optimized sampling

PPV infected: dilutions



Healthy

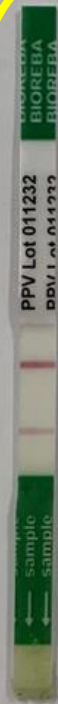
Summary: Optimized sampling

PPV infected: dilutions

1:10



1:40



1:120



Healthy

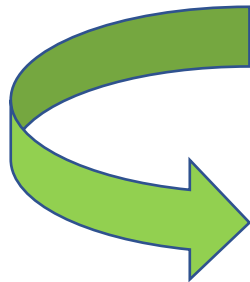
Healthy
1:40



Results from the manufacturers

Validation and optimization

- Detection of **all PPV isolates** (D, M, EA, C (sour and sweet cherry), W, Rec and T strains)
- No **cross-reactivity** with other viruses occurring on the same host plants (example ACLSV / PDV / PNRSV)
- Low/no background with **healthy host** tissue (leaves, petals etc. of *Prunus* sp.)



- This is achieved with:
 - The optimal **extraction buffer**
 - Definition / recommendation for **sample dilution** (w/v). Insist on the max amount of sample

Development & Optimization continue

Extended validation

- Verification with **external partners**:
“reference labs / virus collections etc.”
- **Proficiency test or TPS**:
Valitest (presented by Jean-Philippe Renvoisé)

ABSTRACT

Sensitivity of field tests (AgriStrip and Immunochromato), DAS-ELISA, two step RT-PCR and real-time RT-PCR for *Plum pox virus* (PPV) detection was tested in various tissues of apricot, peach, plum and damson plum trees infected with isolates belonging to PPV-D, PPV-M or PPV-Rec, the three strains present in Slovenia. Flowers of apricot and plum in full bloom proved to be a very good source for detection of PPV.

S Code 1.01

apricots, prunus

Plum Pox

RLIN³

Manufacturer view: Development and Validation: Example PPV AgriStrip

- Selection of antibodies
- Test antibody functionality in LFD format
- Validation: Specificity / Sensitivity / Sampling optimization
- **Definition of the “Scope: Intended Use”**
- Production and standardization: lot-to-lot consistency
- Customer training

Definition of the Scope / Intended use

Based on the results: development / validation / optimization

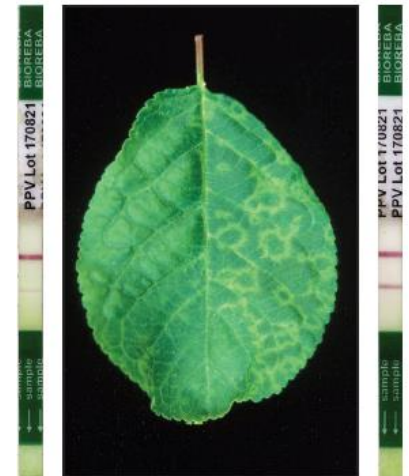


Definition of the scope of the test / Intended use



Results PPV AgriStrip:

- Fresh tissue in the recommended test season
- Symptomatic tissue
- Extraction in extraction buffer B
- Optimal sample dilution of 1:40 (w/v)

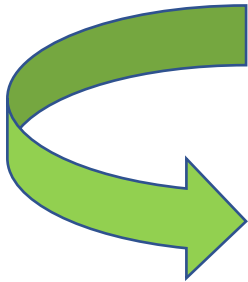


Manufacturer view: Development and Validation: Example PPV AgriStrip

- Selection of antibodies
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- Validation: Specificity / Sensitivity / Sampling optimization
- Definition of the “Scope: Intended Use”
- **Production and standardization: lot-to-lot consistency**
- **Customer training**

Production, Standardization, Customer training

Existing product line: Production and quality control is done according to existing QC-protocols



Criteria that are taken into account:

- Stability tests
- Shelf life
- Storage conditions
- Lot-to-lot consistency

Training of the users: sampling / sample extraction / test



Conclusion: Parameters for a successful test

A validated test



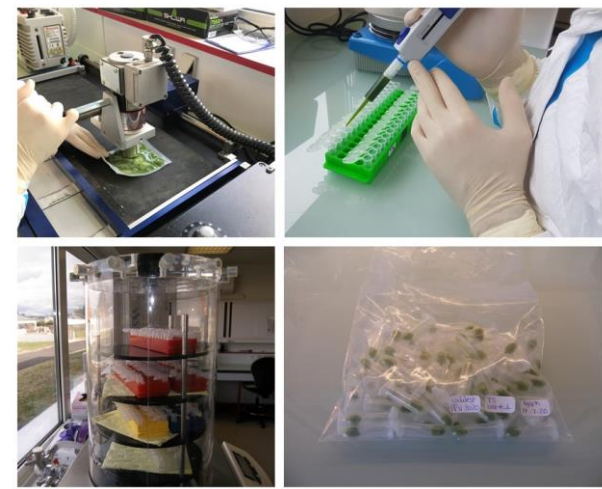
Appropriate sampling



Trained staff



What are the challenges for a successful evaluation by the user?



The use and validation of on-site tests: User view

WEBINAR
2021-01-20

Jean-Philippe Renvoisé
Anses
Plant Health Laboratory
Quarantine unit



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

The use and validation of on-site tests: User view

- Identify performance criteria to be evaluated
- Choose the appropriate evaluation
- Identify parameters that can interfere with the evaluation and validate the modifications made
- Conclude on performance characteristics

Identify performance criteria to be evaluated

→ Based on the **intended use** of on-site tests, identify which performance criteria need to be evaluated

INTENDED USE



EXPECTED PERFORMANCES
FOR ON-SITE TESTS

Detection of
high/medium
target loads

Positive results
for isolates/strains

Negative results
for non-target pests

Negative results
for healthy plants

PERFORMANCE CRITERIA

→ Analytical sensitivity

→ Inclusivity

→ Exclusivity

→ Selectivity

Considered as most
important for on-site tests

Analytical
specificity

Identify performance criteria to evaluate

EXPECTED PERFORMANCES FOR ON-SITE TESTS

Positive results with positive samples

Negative results with negative samples

Confidence in one result per sample

Confidence in results given in different conditions

Confidence in a positive test result

Confidence in a negative test result

PERFORMANCE CRITERIA

→ Diagnostic sensitivity (DSE)

→ Diagnostic specificity (DSP)

→ Repeatability

→ Reproducibility

→ Positive likelihood ratio

→ Negative likelihood ratio

calculated using DSE and DSP

Choose the appropriate evaluation

Organization

In-house evaluation

Internal validation project
e.g. preliminary study of a TPS

Test Performance Study (TPS)

Inter-laboratory evaluation
TPS organizer + participants

Strengths

- . On-site conditions can be respected
- . Complete protocol can be easily evaluated
- . High number of different samples

- . Produce high number of results
- . High confidence in performance characteristics
- . Suitable for reproducibility

Weaknesses

- . Limited number of analytical environments
- . Limited number of operators

- . Limited number of different samples
- . Difficulty to reproduce on-site conditions

SUPPLEMENT EACH OTHER

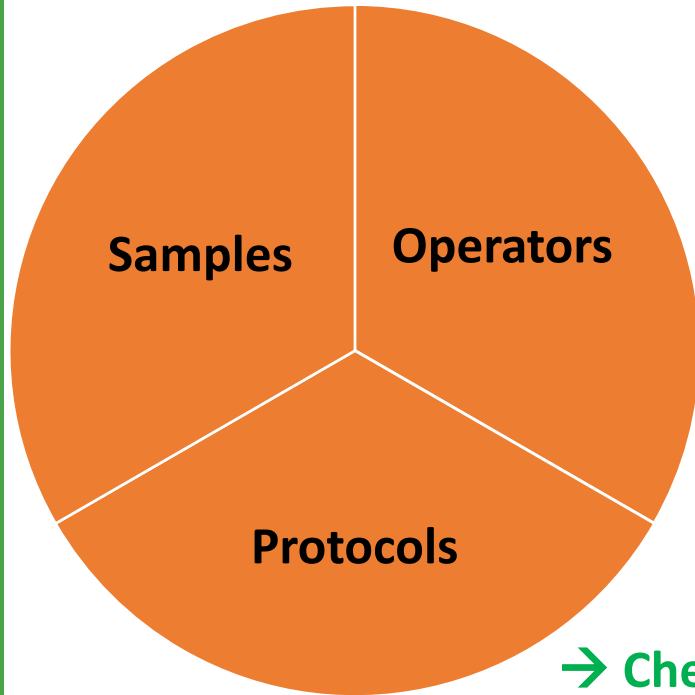
Valitest on-site test evaluations: systematic in-house evaluation + TPS



Identify parameters with possible influence

Validate the modifications

→ Identify parameters that may interfere with the evaluation if analytical environment is too different from on-site conditions



Modifications may be needed

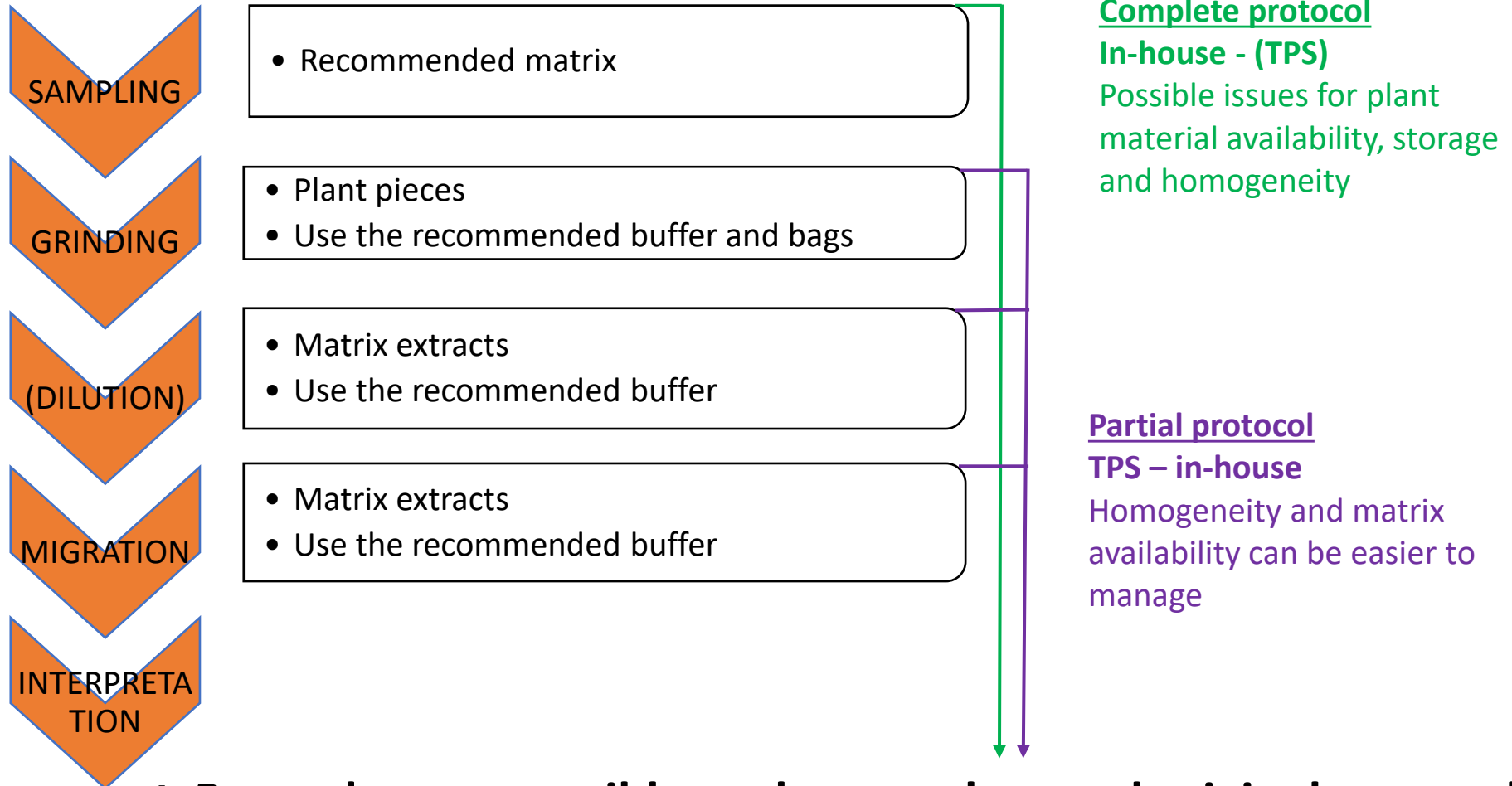
→ Check that the modifications made have no influence

AND / OR

→ Validate the modifications with the manufacturer

Protocols

Example: LFD to be used on fresh symptomatic matrix of host plants



→ Be as close as possible to the complete and original protocol

TPS organizers chose different options depending on the matrix available

Modifications validated by TPS organizers or discussed with manufacturer



Operators

IN-HOUSE

➤ Selection of operators

Give priority to trained operators



➤ Training of operators

Highly recommended
Contact manufacturer

TPS

➤ Selection of participants

Main criteria

- Possibility to participate according to the time schedule
- Quality assurance in place or traceability system in place

On-site tests can be used by users who are not experts with the test/method

Proficiency: not a selection criterion

➤ Training of participants

Difficult to organize
Replaced by more precise instructions given to participants

Samples

FRESH
MATERIAL
AVAILABILITY

→ **Collect and test in the right season or deseasonalize in dedicated facilities**

- easy for in-house evaluation
- difficult for TPS

The best option for on-site tests

→ **Alternative solutions: collect and store and check for potential matrix effect**

Different storage solutions: freeze, freeze-dry, extract nucleic acids,...

Valitest TPS organized in winter
TPS organizers chose different options for storage

Example: Evidence of a matrix effect

- Use of freeze-dried plant material ground into fine powder
- Preliminary study results impacted by the matrix preparation
- Protocol adjustments could not be validated due to unavailability of fresh material
- Agreement of manufacturer on the protocol adjustments made
- In-house evaluation using fresh material organized to confirm the TPS results



Samples

COLLECT THE
RECOMMENDED
MATRIX

→ Avoid potential matrix effects

COLLECT WITH
SYMPTOMS

→ For target and non-target pests

→ Collect in the best period



Example: Unavailability of symptomatic plants

- *Asymptomatic or poorly symptomatic plants available for non-target pests*
- *Used for preliminary study only to check for potential cross-reactions - not used in the TPS*
- *Results to be confirmed with fresh plant material*

Samples

USE
ASYMPTOMATIC
PLANT
MATERIAL

USE DILUTIONS

→ « Healthy » plant material used for selectivity assessment

→ Dilutions used for analytical sensitivity assessment

- If symptomatic plant material is required:
 - . Prepare moderate dilutions
 - . Prepare specific panels for on-site tests
- If symptomatic plant material is not required:
 - . Possibly higher dilutions can be prepared



Samples

HOMOGENEITY / ASSIGNED VALUE

- High limit of detection for most on-site tests
- Possible uneven distribution of the pest in the matrix

Homogeneity: a crucial point to be met for on-site tests !

Example: How to provide enough homogeneous plant material for all participants ?

- *Use of plant extracts spiked with different isolates instead of fresh material*
- *Confirmation in the preliminary study that suggested modification didn't affect the results and that extracts were homogeneous and stable.*
- *Agreement of manufacturer on the modifications made*

More information on “**Preparation and dispatch of samples**”:
webinar #4 of the series on Test Performance Studies organisation
https://www.valitest.eu/training/activities_and_webinars

- Test sample lots for homogeneity
- Determine the assigned value for samples
- Choose homogeneous sample lots for panels constitution

Samples

PANEL OF SAMPLES

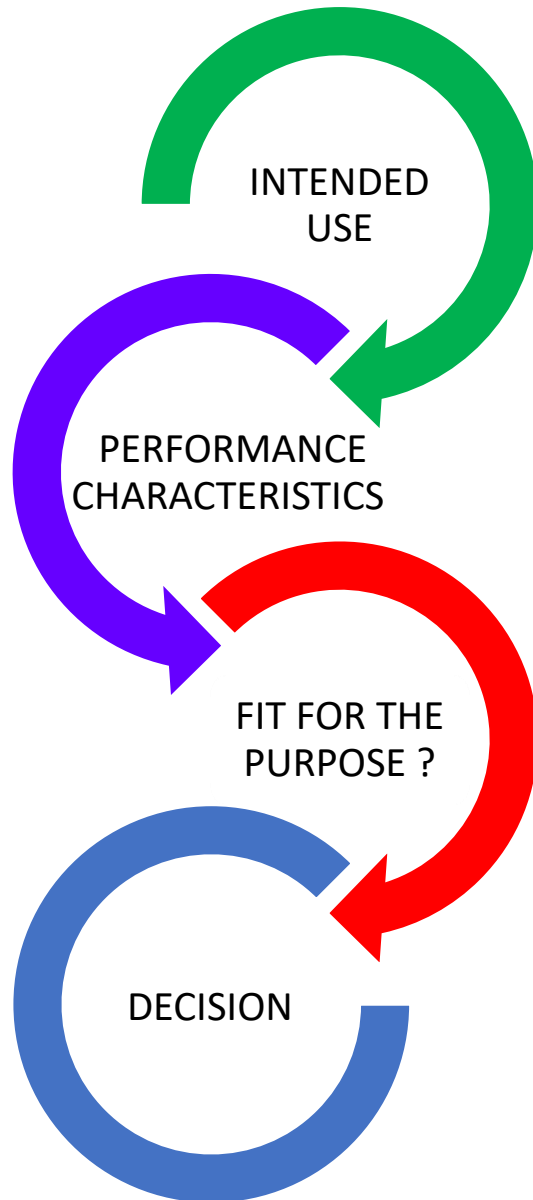
- According to the performance criteria chosen
- According to the results of the homogeneity study



STABILITY

- Store dedicated samples in the same conditions as for participants (TPS)
- Set a short term deadline for analyzing samples
- Test with the same method as for homogeneity
- Choose stable lots for statistical analysis

Conclude on performance characteristics



Fresh plant material
Symptomatic or not
Host plant

Production of validation data

Context to be taken into account

Criteria to be evaluated in the field conditions

- speed of execution
- ease of implementation
- traceability of the test result

and other criteria such as cost and level of training for operators

TAKE HOME MESSAGES



→ COLLABORATE WITH MANUFACTURER FOR MANY REASONS:

- Fully understand the protocol (samples, implementation, interpretation, troubleshooting)
- Ask for more information
- Ask for same lot number of reagents for validation
- Discuss about abnormal results
- Ask for advices in case of modification of the protocol



TAKE HOME MESSAGES

→ BE AS CLOSE AS POSSIBLE TO THE ON-SITE CONDITIONS

- Matrix
- Seasons
- Symptoms
- Protocols

Thank you for your attention!

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