Introduction of the webinar and training activities

Test Performance Studies organisation

Videos	What is a TPS?	On the week 02/15
Videos	VALITEST TPS: selection of the pests and of the TPS organizers	On the week 02/15
Webinar 1	Preparing the TPS plan	Friday 19/02, 11am
Webinar 2	Selection of the tests and associated documents	Wednesday 24/02, 2pm
Webinar 3	Selection of participants and contract	Monday 1/03, 2pm
Webinar 4	Preparation and dispatch of samples	Friday 5/03, 11am
Webinar 5	Production of reference material for TPS	Wednesday 10/03, 2pm
Practical training sessions	How to organise Test Performance Studies?	15-17/03 (3 sessions)
Webinar 6	**How to tackle the analysis of TPS results?**	**Monday 22/03, 2pm**
Videos	Calculate performance characteristics of a test and get useful information from your validation data by statistical analysis.	On the week 22/03
Webinar 7	Q&A session: the statistical analysis of TPS results	Monday 29/03, 2pm
Practical training sessions	How to analyse the results of Test Performance Studies?	30-31/03 (2 sessions)
Webinar 8	From TPS organisation to analysis of the results: example of the TPS on ToBRFV	Wednesday 7/04, 2pm
Videos	Reporting TPS results	To be confirmed/announced

VALITEST webinar series and training activities

How to tackle the analysis of TPS results

22nd March 2021

Jenny Tomlinson (Fera Science Ltd)

Q & A with TPS organisers M. Luigi (CREA), M. Mezzalama (UNITO)

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

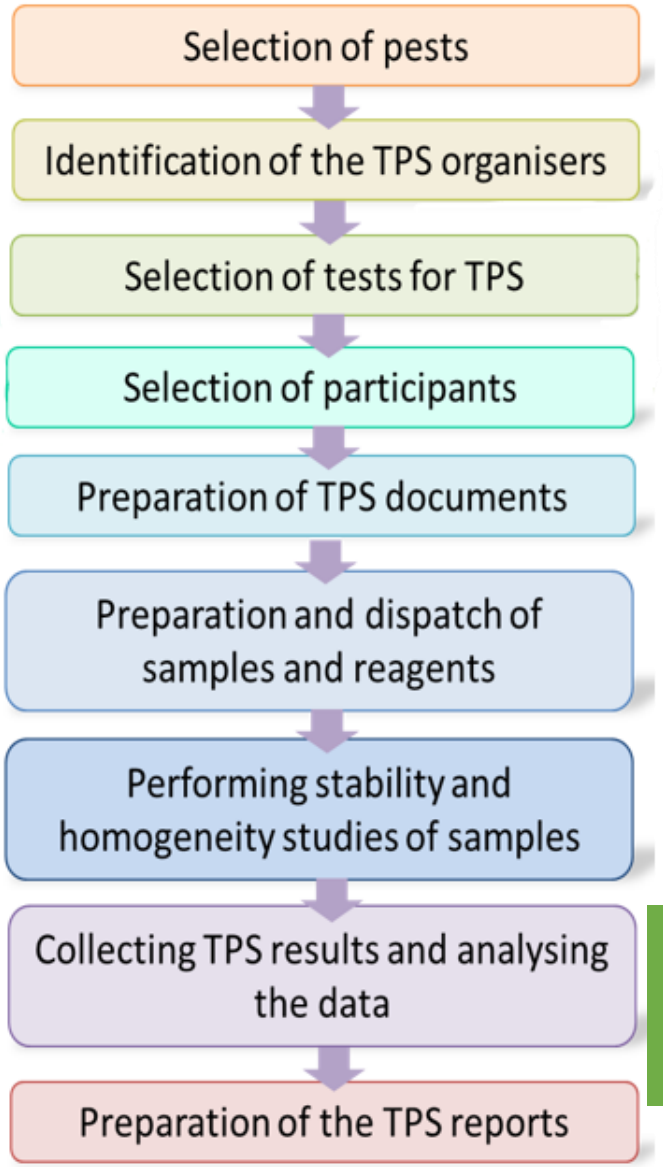
Outline of the presentation

- Introduction
- Extraction of results from results sheets
- Inconclusive results
- Exclusion of results from analysis - controls
- Exclusion of results from analysis – deviations from protocol

- Interviews of TPS organisers (video)

- Q & A

TPS organization workflow



Webinar 6 22nd March
Videos starting 22nd March and **Q&A session** 29th March
Practical training sessions 30th-31st March

Valitest

Question to the audience

Have you already performed an analysis of TPS results?

- Yes
- No

Valitest

Analysis of TPS results

Analysis of the results can be one of the most challenging aspects of the TPS…

- Extraction of data from the results sheets submitted by participants
- Consideration of the validity of the results – are they suitable for inclusion in the analysis?
- Statistical analysis
- Interpretation of the results

Sample panel to include positive and negative samples and a serial dilution to allow calculation of:

- **Analytical sensitivity** (probability of detection) – dilution series
- **Repeatability** (accordance) and **reproducibility** (concordance) – all samples
- **Diagnostic sensitivity** and **specificity** (and measures derived from them e.g. **likelihood ratios**) – independent samples

Valitest

Analysis of TPS results

Analysis of the results can be one of the most challenging aspects of the TPS…

- Extraction of data from the results sheets submitted by participants
- Consideration of the validity of the results – are they suitable for inclusion in the analysis?
- Statistical analysis
- Interpretation of the results

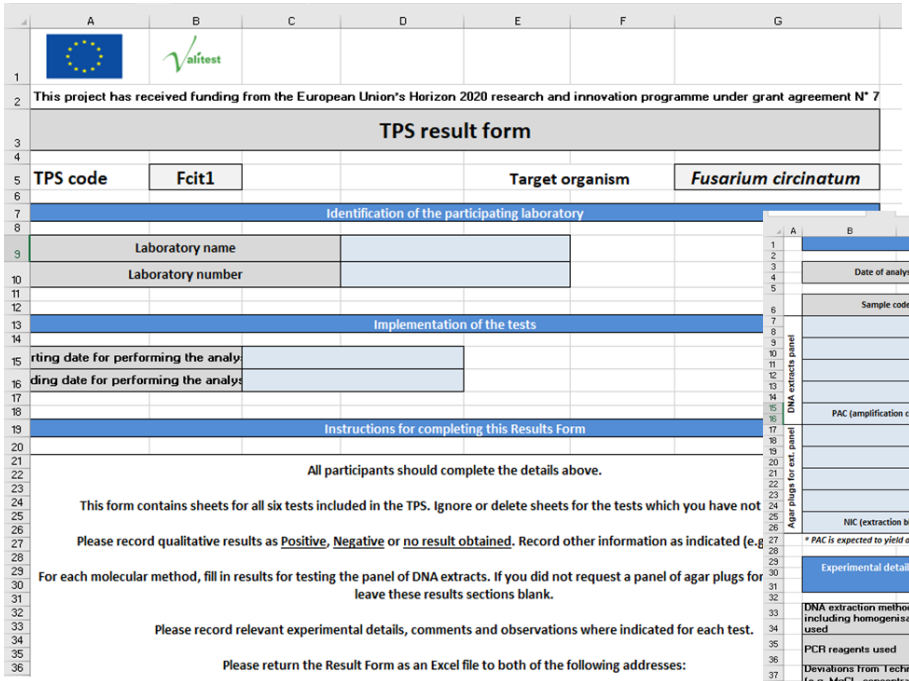
see videos on the specific methods at:
www.valitest.eu/training/activities_and_webinars

Sample panel to include positive and negative samples and a serial dilution to allow calculation of:

- **Analytical sensitivity** (probability of detection) – dilution series
- **Repeatability** (accordance) and **reproducibility** (concordance) – all samples
- **Diagnostic sensitivity** and **specificity** (and measures derived from them e.g. **likelihood ratios**) – independent samples

Valitest

Extraction of data from results sheets



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 7

TPS result form

TPS code	Fcit1	Target organism	*Fusarium circinatum*

Identification of the participating laboratory

Laboratory name	
Laboratory number	

Implementation of the tests

rting date for performing the analy	
ding date for performing the analys	

Instructions for completing this Results Form

All participants should complete the details above.

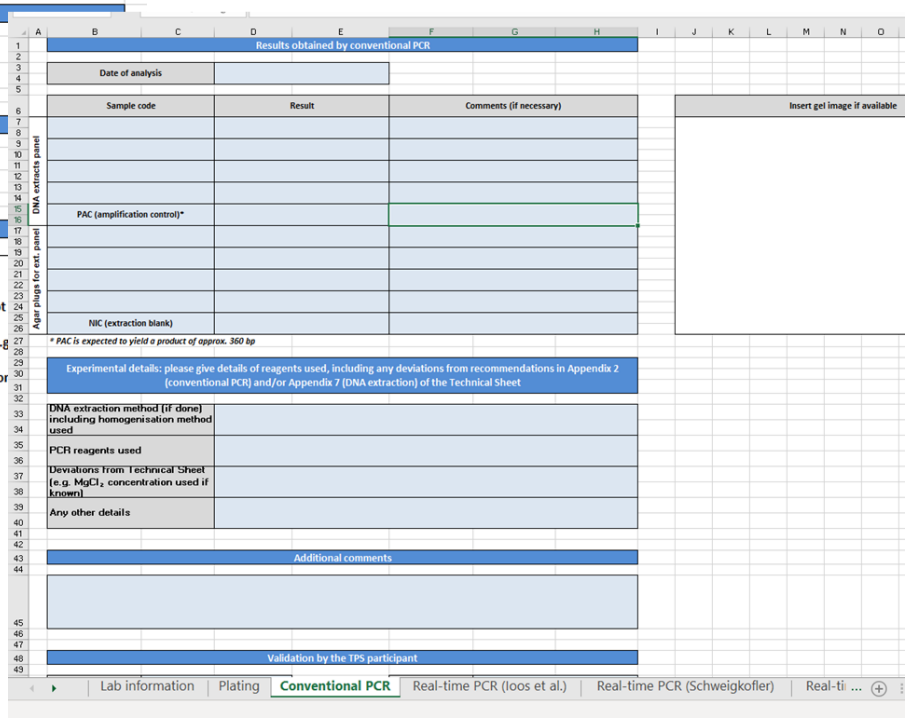
This form contains sheets for all six tests included in the TPS. Ignore or delete sheets for the tests which you have not

Please record qualitative results as Positive, Negative or no result obtained. Record other information as indicated (e.g

For each molecular method, fill in results for testing the panel of DNA extracts. If you did not request a panel of agar plugs for leave these results sections blank.

Please record relevant experimental details, comments and observations where indicated for each test.

Please return the Result Form as an Excel file to both of the following addresses:



Results obtained by conventional PCR

Date of analysis	

	Sample code	Result	Comments (if necessary)
DNA extracts panel			
	PAC (amplification control)*		
Agar plugs for ext. panel			
	NIC (extraction blank)		

Insert gel image if available

** PAC is expected to yield a product of approx. 360 bp*

Experimental details: please give details of reagents used, including any deviations from recommendations in Appendix 2 (conventional PCR) and/or Appendix 7 (DNA extraction) of the Technical Sheet

DNA extraction method (if done) including homogenisation method used	
PCR reagents used	
Deviations from Technical Sheet (e.g. $MgCl_2$ concentration used if known)	
Any other details	

Additional comments

Validation by the TPS participant

Lab information | Plating | Conventional PCR | Real-time PCR (Ioos et al.) | Real-time PCR (Schweigkofler) | Real-ti ...

Extraction of data from results sheets

- Data returned by participants is likely to be complex
  - Test results (positive, negative, inconclusive/undetermined): ≥ 200 data points per test
  - Need to deconvolute randomisation imposed on the samples
- Format for return of results should be planned with this in mind – e.g. Excel spreadsheet, formatted to allow easy extraction of data
- Additional information: tests for plant pests are often treated as qualitative tests (+/-), even if they provide a quantitative measurement, but TPS organisers may also request raw data (measurements, amplification plots, gel pictures) to allow additional scrutiny of results

Valitest

Preparing data for analysis

First need to ensure that data is suitable for analysis…

Question to the audience

Do you think that it is important to exclude results from the analysis?

- Yes, only valid results should be included in the analysis
- No, all results should be taken into account
- Sometimes – it depends on the context

Valitest

Inconclusive results

- Some tests only have two possible outcomes (positive or negative) – e.g. LFD read by eye
- For other tests, a third result (inconclusive) may be possible

- Tests which generate quantitative data but are treated qualitatively may have a defined grey area e.g. Ct values where a cut-off is applied above which the sample is not called as positive or negative
- The Ct cut-off value that should be used is dependent on equipment, material, chemistry (EPPO diagnostic protocols)
- If a participant imposes a cut-off in the interpretation of their results, this information should be provided (and probably raw data too)

- A result may also be called inconclusive if e.g. replicate reactions give contradictory results

Valitest

Inconclusive results

- TPS organiser may instruct participants to repeat testing in the event of an inconclusive result (but there may not be enough sample/reagents/time to repeat more than once)
- If it is still not possible to obtain a positive or negative result, this could be treated in the same way as a missing result for the analysis i.e. no result, rather than a false result

- i.e. 'inconclusive' = no result = exclude from the analysis…

- BUT the tendency of a test to fail or generate an uninterpretable result is an important observation (vs results missing e.g. due to error)

- In the event of a missing or inconclusive result, the TPS organiser may choose to omit the whole data set (e.g. if missing result(s) makes it impossible to determine whether the data set is valid) or just the individual result

Valitest

Exclusion of results from the analysis

- Need to ensure that data is suitable for analysis – identify outliers
- Statistical approaches to identification of outliers
- Technical considerations for identification of data not suitable for inclusion in the analysis – depends on expertise and judgement of the TPS organiser

1. Exclusion of data due to the results obtained for **controls**

Positive and negative controls are included in all tests to allow interpretation of results

2. Exclusion of data due to **deviations from the protocol**

Some deviations may be acceptable while others may invalidate the results; some potential deviations may be anticipated by the TPS organiser

Valitest

Failure of positive controls

- Positive control / positive isolation control (PIC)/ positive amplification control (PAC) - to allow interpretation of negative results
  - Expected to be positive
  - Positive sample to be isolated e.g. infected material for DNA extraction followed by PCR (PIC)
  - Nucleic acid expected to give a positive result e.g. amplification by PCR (PAC)

- Negative result indicates failure of test/extraction/amplification
  - Operator error – all samples or sporadic?
  - Sample degradation – all samples or sporadic?
  - Reagent degradation

Valitest

Failure of negative controls

- Negative control / negative isolation control (NIC)/ negative amplification control (NAC) – to allow interpretation of positive results
  - Expected to be negative
  - Negative sample to be isolated e.g. healthy material for DNA extraction followed by PCR (NIC)
  - Material expected to give a negative result e.g. amplification by PCR (NAC)

- Positive result indicates failure/contamination of test/extraction/ amplification
  - Operator error – all samples or sporadic?
  - Sample contamination – all samples or sporadic?
  - Reagent degradation? – raw data may give an indication

Valitest

Failure of controls

- Data set for each test consists of results for controls and samples
- Exclude data set if the results for any of the controls are incorrect (false negative/positive, inconclusive) or if the control was omitted for any reason, consider all results in the data set as inconclusive i.e. in the absence of correct results for the controls, the results could not be interpreted reliably)
- Useful to compare how many valid data sets were obtained for each test
- Exclusion of data may be straightforward or may require nuanced interpretation by the TPS organiser

Valitest

To include or exclude… failure of controls

- Failure of control(s) for one test may indicate that the test is particularly sensitive to variables that are not ordinarily controlled… i.e. lacks reproducibility
- We don't want to ignore results which demonstrate a lack of reproducibility…
- Only considering the data sets with concordant controls could obscure the lack of reproducibility
- May need to draw conclusions based on the exclusion of data as well as the analysis of valid data

Valitest

To include or exclude… failure of controls

- May be exacerbated by varying levels of expertise in participating labs…
- If only highly experienced labs participate, or if the results from less experienced labs are more likely to be excluded from analysis, we will only produce performance data for the test as carried out in a very experienced lab. (Note: This might be what we want to know!)
- We might actually want to know how the test performs in a range of labs with a reasonable level of relevant experience, not just those with the highest level of expertise
- This may be particularly relevant for on-site methods…

On-site tests and reproducibility

- Laboratory tests – reasonable expectation of a high level of technical proficiency in labs carrying out testing
- May have different expectations of tests to be used outside the laboratory
- It may not be particularly useful to only know about the performance of on-site tests when they are carried out in a laboratory and by staff with specialist technical expertise
- May need to take this into consideration when planning the TPS (selecting participants) and/or when analysing the results and drawing conclusions from the analysis
- Who the end users are is very context dependent – caveat performance data obtained in the lab with recommendations that additional work may be necessary to understand performance in specific scenarios

Valitest

To include or exclude: thought experiment…

- Example: a test with extremely *high analytical sensitivity* but also *high operational complexity* may be more susceptible to failed negative controls caused by contamination.
- Contamination risk associated with particular labs (less experience, different equipment etc).
- TPS organiser may exclude data sets from labs with failed negative controls
  - ➢ also excludes false positive results
  - ➢ increases the estimated reproducibility for the test*
- But low reproducibility caused by high susceptibility to contamination is an important observation to make about this test. The important thing is to document this conclusion in the TPS report.

*also specificity, positive likelihood ratio etc

Valitest

Deviations from protocol

Decisions made in the design of the TPS will affect how likely participants are to deviate from protocols…

Question to the audience

As a TPS organiser, I would prefer to provide:

- All the required reagents for a test
- Specific reagents only (e.g. primers but not polymerase for PCR; antibodies but not buffers for ELISA etc)

- Completely prescriptive instructions
- Some flexibility to suit individual laboratories

Valitest

Question to the audience

As a participant in a TPS, I would prefer to receive:

- All the required reagents for a test
- Specific reagents only (e.g. primers but not polymerase for PCR; antibodies but not buffers for ELISA etc)

- Completely prescriptive instructions
- Some flexibility to suit my own laboratory

Valitest

Deviations from protocol

- Some tests, especially commercial kits, may offer very little chance to deviate from protocol e.g. (lateral flow devices)
- For other tests, it depends on how prescriptive the instructions are – this is often a judgement to be made by the TPS organiser

All reagents provided, all variables controlled

Participants choose and source own reagents, make own choices about some variables (e.g. instrument/ model of thermal cycler)

Valitest

Deviations from protocol – which reagents should the TPS organiser provide?

- TPS organiser may choose to supply some/all reagents for some/all tests

Reagents provided by TPS organiser

- Reduces chance of deviations
- Cost to the TPS organiser
- Logistics for distribution
- Risk of degradation during shipping

Reagents provided by participants

- Cost to participants
- Labs may choose not to participate due to cost (need ≥10 labs to take part)
- Difficulties in obtaining reagents may cause delays

Valitest

Deviations from protocol – how prescriptive should the instructions be?

- TPS organiser may choose to specify, for example, use of a specific instrument/ model, reagents, homogenisation/DNA extraction method etc
- TPS organiser may carry out preliminary testing to assess which deviations might invalidate results and inform participants of acceptable deviations
- May need to make pragmatic decisions – if every detail is controlled, there may be too few participants

More prescriptive instructions

- Labs may not be able to participate (e.g. if they do not have specified model of instrument)
- Labs may choose not to participate (e.g. if they favour reagents which are not specified)
- **Less chance that results will deviate from expectations**

Less prescriptive instructions

- A more realistic picture of the performance of tests in the range of labs that will use them
- **More chance that results will deviate from expectations**

Valitest

Deviations from protocol – how prescriptive should the instructions be?

- Examples: real-time PCR

- Many reagent options (e.g. core reagents vs master mixes), availability may differ in different countries…

  - Make recommendations based on results of preliminary testing – more than one option if possible
  - Define and control the key variable for participants unable to use the recommended reagents – Mg^{2+} concentration; passive reference; compatibility with instrument used etc
  - Instruct participants to record any deviations from the instructions

To include or exclude… deviations from protocol

- Results should be excluded if they have been adversely affected by deviation from the protocol (intentional or inadvertent)

- This may mean that the protocol can be redefined to be made more stringent (e.g. ‘use of polymerase X with these primers will cause suboptimal performance of this test’)

- Examination of the information provided by participants may allow a protocol to be made less prescriptive (e.g. expanding the list of polymerases which can be used successfully)

Valitest

Interviews – TPS organisers

“What was the major difficulty you encountered when analysing the TPS results (outliers, missing results, performance criteria assessment, …)? How did you circumvent this difficulty?”

“How did VALITEST and dedicated WPs help you with the analysis of the TPS results?”

TPS organisers' experiences: lessons learned

- Preparing data for analysis can be challenging
- Automation of data extraction and analysis could increase efficiency
- Identification of outliers and decision to exclude data is not straightforward
- Additional information provided by participants can inform these decisions (deviations from protocols, inconsistency of interpretation)

- Outputs from Valitest (Work Package 2 recommendations) and discussion with other WP organisers provided clarity and focus, as well as consistency

Interpretation of the TPS results

- How do I want to use the results of the TPS?
  - To inform the choice of a test from the options available
    - there will be many other factors to consider in addition to the performance characteristics of each test (cost, availability, time, staff, facilities etc etc)
    - selection may differ for different testing scenarios (TPS results may indicate which test(s) are most suitable for particular uses)
  - Identify shortcomings to inform development of new tests
  - Understand uncertainty associated with the results of tests
  - Understand how tests could be used in combination to achieve particular outcomes

Valitest

Summary

- The ability to determine the validity of data sets, and the ease of handling the data, often relies on decisions made earlier in the TPS planning process, for example:
  - TPS scope and/or selection of participants
    - May influence treatment of outliers
  - Choice of controls
    - Will affect inclusion/exclusion of data
  - Preparation of instructions and technical sheets
    - Will affect how likely participants are to deviate from protocols
  - Design of results forms
    - Opportunity to capture additional information
    - Allow more efficient/automated extraction of data

- Careful consideration of the validity of participant's data will ensure robust conclusions can be made on the basis of the statistical analysis

Valitest

Thank you for your attention!

E-mail: jenny.tomlinson@fera.co.uk

Valitest

Introduction of the webinar and training activities

Test Performance Studies organisation

Videos	What is a TPS?	On the week 02/15
Videos	VALITEST TPS: selection of the pests and of the TPS organizers	On the week 02/15
Webinar 1	Preparing the TPS plan	Friday 19/02, 11am
Webinar 2	Selection of the tests and associated documents	Wednesday 24/02, 2pm
Webinar 3	Selection of participants and contract	Monday 1/03, 2pm
Webinar 4	Preparation and dispatch of samples	Friday 5/03, 11am
Webinar 5	Production of reference material for TPS	Wednesday 10/03, 2pm
Practical training sessions	How to organise Test Performance Studies?	15-17/03 (3 sessions)
Webinar 6	How to tackle the analysis of TPS results?	Monday 22/03, 2pm
Videos	**Calculate performance characteristics of a test and get useful information from your validation data by statistical analysis.**	**On the week 22/03**
Webinar 7	**Q&A session: the statistical analysis of TPS results**	**Monday 29/03, 2pm**
Practical training sessions	**How to analyse the results of Test Performance Studies?**	**30-31/03 (2 sessions)**
Webinar 8	From TPS organisation to analysis of the results: example of the TPS on ToBRFV	Wednesday 7/04, 2pm
Videos	Reporting TPS results	To be confirmed/announced