

Introduction of the webinar series

The use and validation of High Throughput sequencing (HTS) tests for diagnostics of plant pests.

Webinar 1	What is High Throughput Sequencing (HTS)?	Friday 30 th April, 2 pm-3:30 pm
Webinar 2	How to prepare your laboratory to conduct HTS tests?	Monday 3 rd May, 2 pm-3 pm
Webinar 3	How to develop, validate and routinely use HTS for diagnostic purpose?	Tuesday 4 th May, 2 pm-3:30 pm
Practical training activity	How to apply the guidelines to your laboratory?	Wednesday 5 th May, 2 pm to 4:30 pm. Friday 7 th May, 2 pm to 4:30 pm.





Adopting HTS technologies in a laboratory

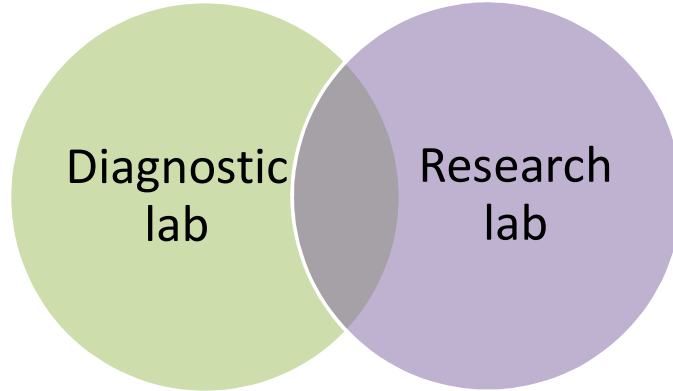
Sebastien Massart





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

1. Applying HTS in a laboratory?



Keep in mind these two aspects and think about:
which steps are mandatory for research lab ?

1. Applying HTS in a laboratory?



Shotgun
Mix

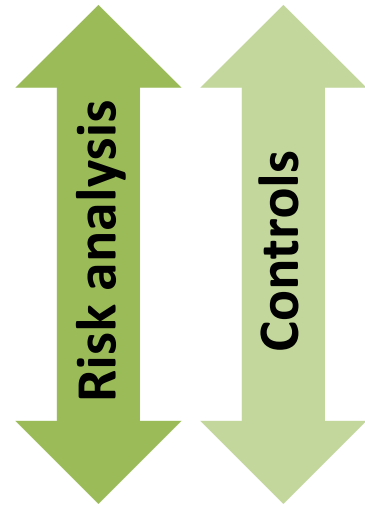
Amplicon
Mix

Shotgun
Pure

1. Applying HTS in a laboratory?



- A. Selection
- B. Development / Optimization
- C. Validation
- D. Verification
- E. Change management
- F. Routine use



2. Selection of a HTS test



- ▶ The HTS tests should be selected according to their intended use (which pest/matrix), the laboratory constraints and the availability of validated tests

- ▶ For both laboratory and bioinformatics



2. Selection of a HTS test

- ▶ Which HTS test would you use in several situations ?

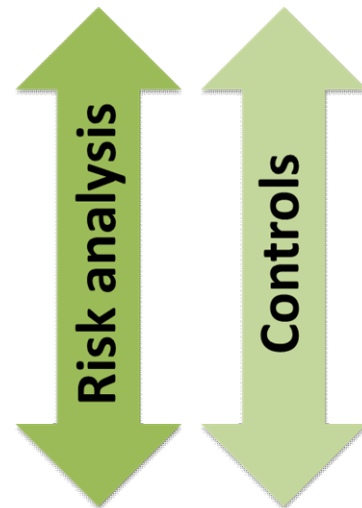


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1. Applying HTS in a laboratory?

- A. Selection
- B. Development / Optimization**
- C. Validation
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3. Development/optimisation of a HTS test



- ▶ **If** no official HTS standard protocol
- ▶ From published HTS test to “ready-for-validation” test

3. Development/optimisation of a HTS test



- ▶ gaining the necessary **experience** with the test by identifying the critical steps, parameters and quality metrics that may affect the test performance;
- ▶ defining the most **appropriate controls** and their continuous monitoring strategy;
- ▶ establishing the **quality metrics thresholds** and acceptable ranges;
- ▶ Preparing/adapting a **detailed protocol** describing the optimized test conditions and analysis settings for validation and subsequent routine use.

3. Development/optimisation of a HTS test



▶ Two key elements to keep in mind:

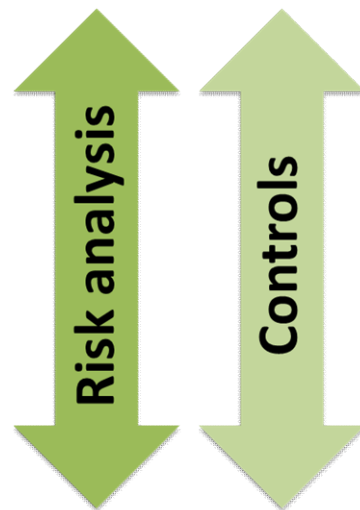
- Sample panel:
 - › reference material
 - › Representativeness of targets
 - › Concentration

- Level of pooling of samples

1. Applying HTS in a laboratory?



- A. Selection
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4. Validation of a HTS test

▶ ISO 9000 (2015)

“confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled”

▶ ISO 17025 (2017)

“the validation shall be as extensive as is necessary to meet the needs of the given application or field of application”



4. Validation : ANALYTICAL SENSITIVITY

▶ **Generated reads**

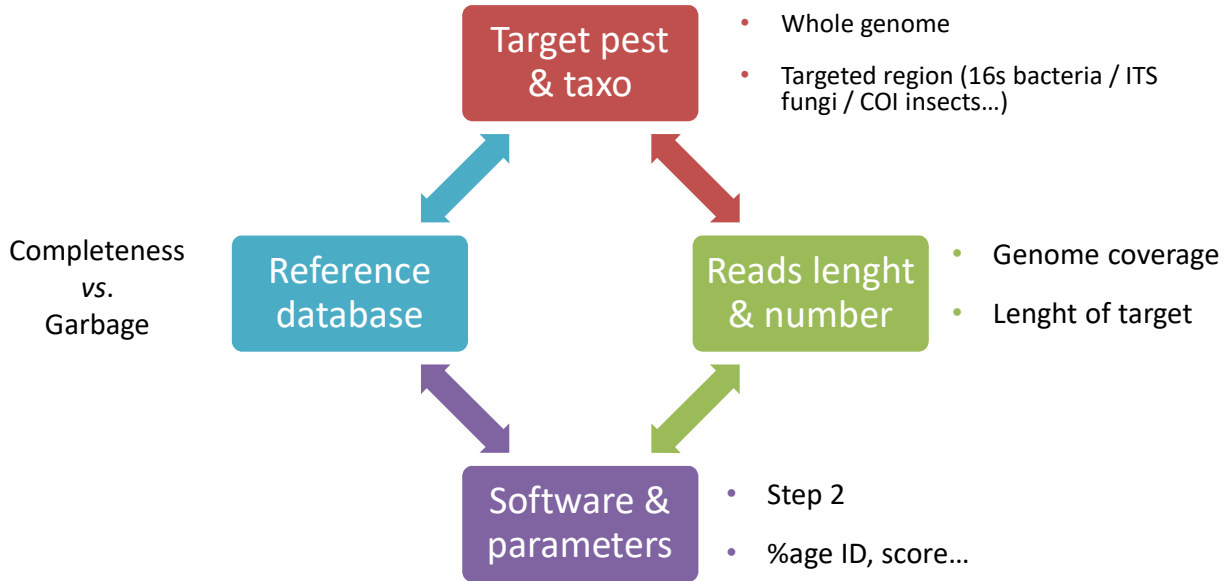
- The 1:50000 ratio
- The contamination

▶ Minimal sequence number

▶ **Multiple targets**

- Competing inside the plant
- Competing for reagents (NTC case)

4. Validation : ANALYTICAL SPECIFICITY



4. Validation of a HTS test



- ▶ Selectivity: internal controls
- ▶ Repeatability & reproducibility: EPPO standard
- ▶ How to adapt them ?
- ▶ Focus of the workshop Wed & Fri



4. Validation of a HTS test

- ▶ Brainstorming among participants
- ▶ Small groups -> cases for validation
 1. Shotgun sequencing for virus detection
 2. Amplicon sequencing for bacteria/fungi detection
 3. Shotgun sequencing for genome characterization



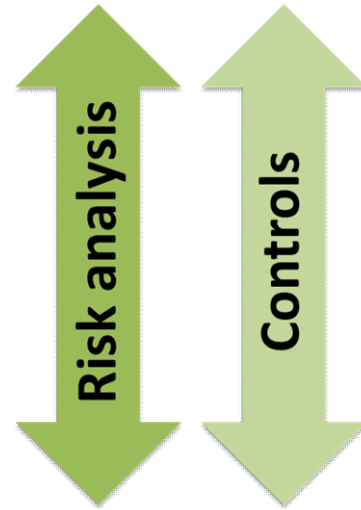
4. Validation of a HTS test

- ▶ Heavy to carry out
- ▶ Banana germplasm viral indexing:
 - >100 accessions
 - All controls
 - Repeatability & reproducibility
 - Dilution series

1. Applying HTS in a laboratory?



- A. Selection
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5. Verification of a HTS test



- ▶ When a existing standard validated HTS test exist
- ▶ Can the laboratory perform the validated test according to established performance criteria ?



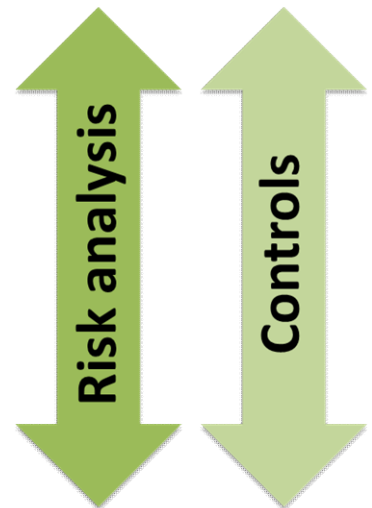
5. Verification of a HTS test

- ▶ EPPO standard PM7/98 guidelines:
 - Analytical sensitivity
 - Analytical specificity (inclusivity & exclusivity)
 - Selectivity
 - Repeatability
 - Reproducibility
- ▶ « Light validation »



1. Applying HTS in a laboratory?

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6. Change Management



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Accueil > Actualités > DNAVision(Gosselies, Belgium) among the first in Europe to receive the ISO 17025 accreditation of exome sequencing service for clinical applications.

t

DNAVision(Gosselies, Belgium) among the first in Europe to receive the ISO 17025 accreditation of exome sequencing service for clinical applications.

An external audit carried out by the Belgian Authorities recently validated DNAVision's (Gosselies, Belgium) technical competence to carry out exome sequencing* and the ability to consistently produce valid and traceable results. This important validation will allow DNAVision to act as pioneer in applying this exome sequencing technology to medical practice.

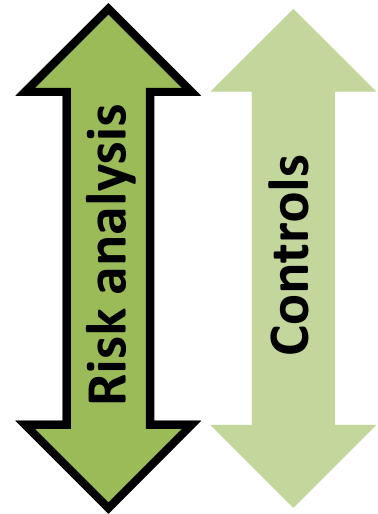
6. Change Management



- ▶ HTS are evolving technologies
- ▶ Evaluate the impact of any change in the laboratory or for the bioinformatic analysis
- ▶ OK <-> Verification <-> Validation

1. Applying HTS in a laboratory?

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7. Risk analysis

- ▶ What are the risk of HTS testing ?

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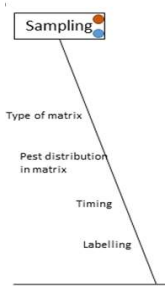
- ▶ Ishikawa & 6M



Result of the pool

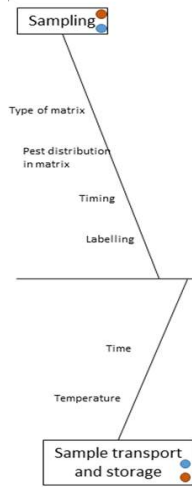


7. Risk analysis

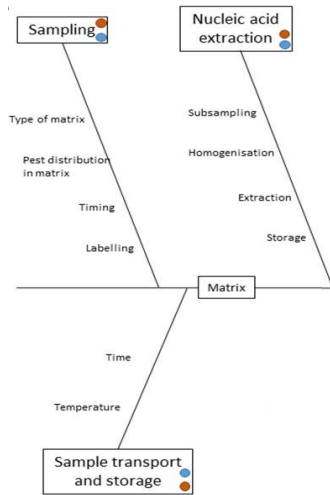




7. Risk analysis

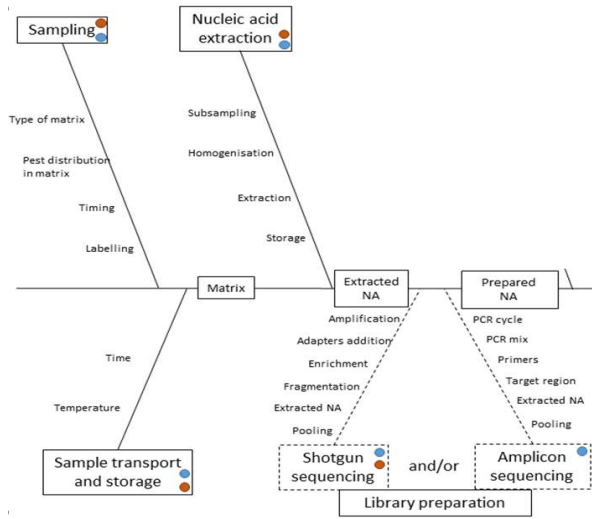


7. Risk analysis

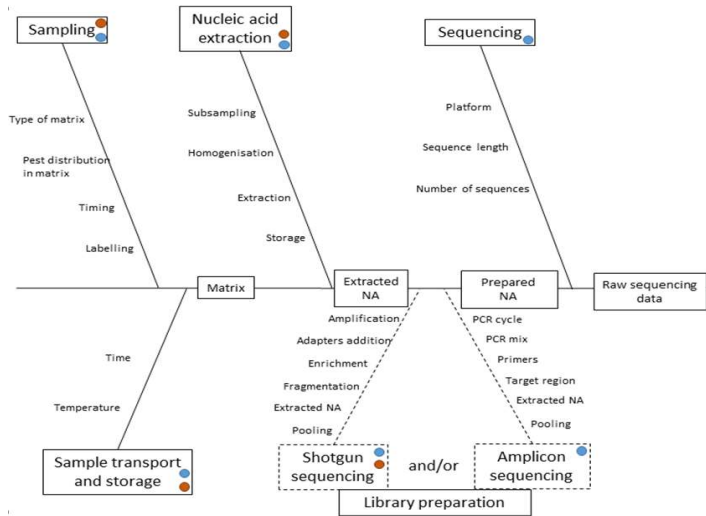




7. Risk analysis

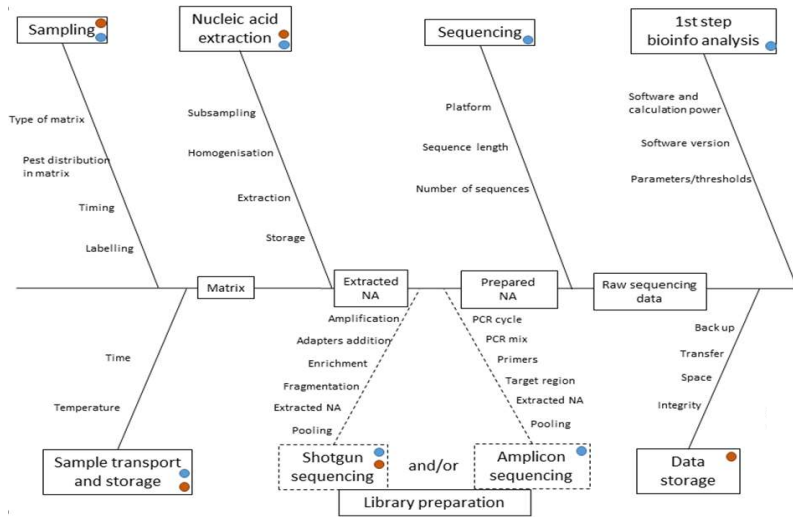


7. Risk analysis

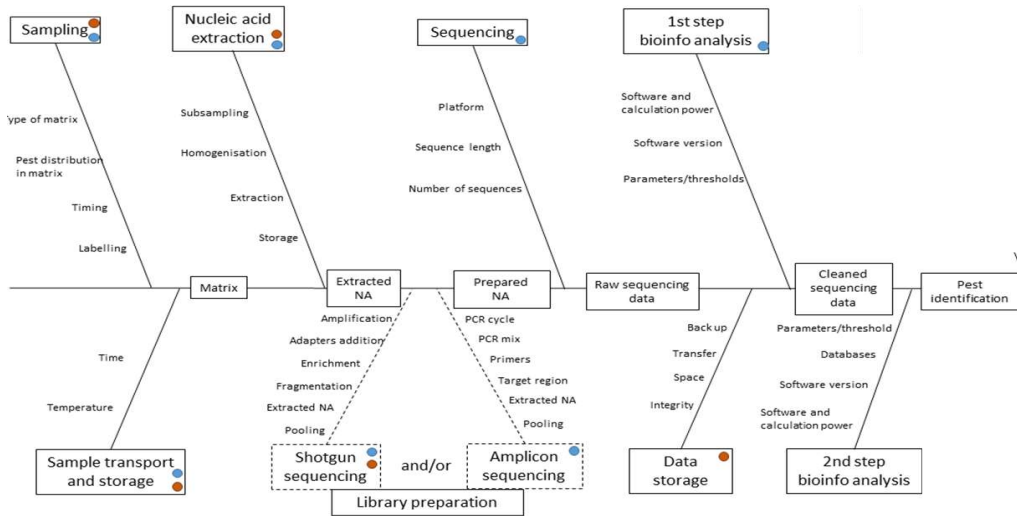




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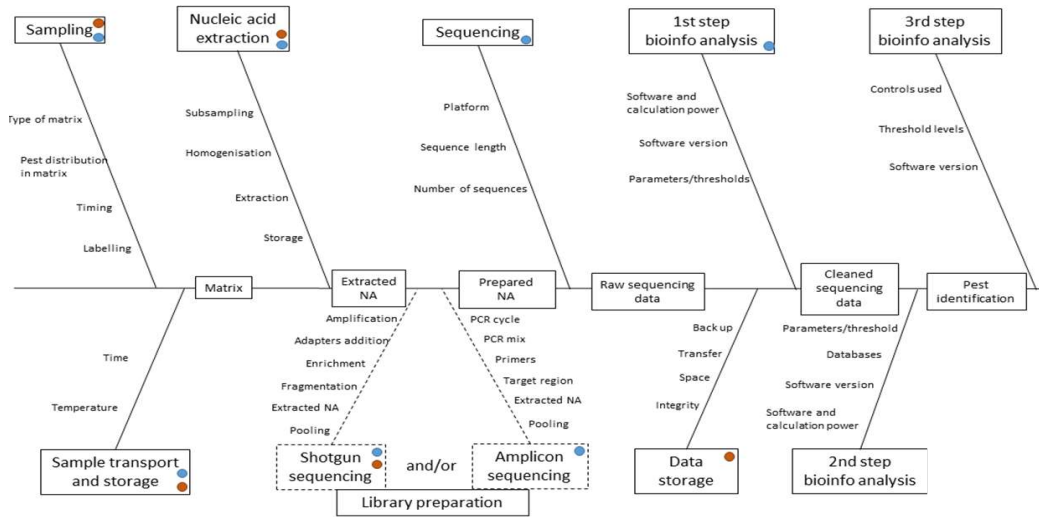


7. Risk analysis

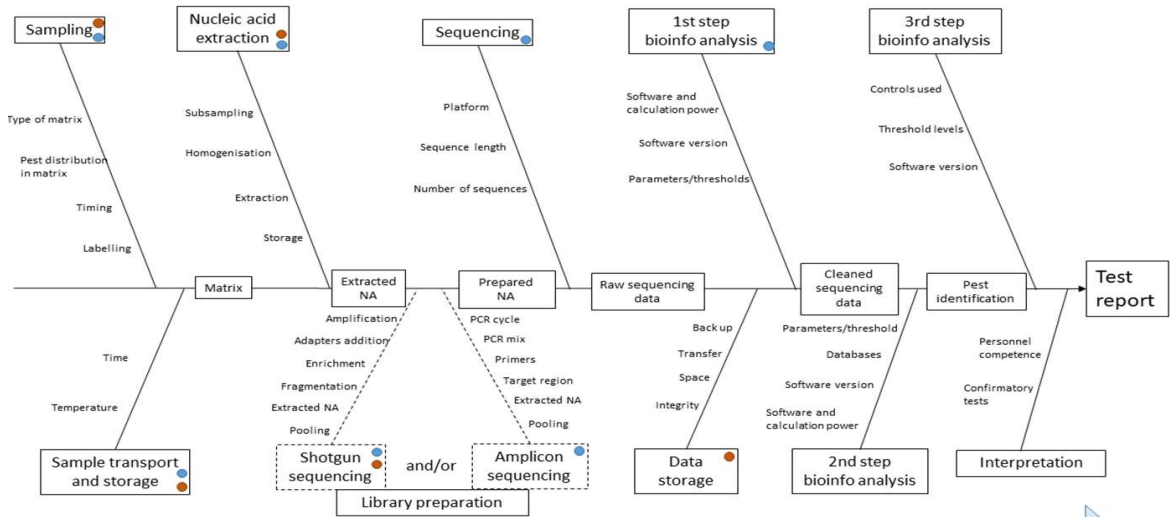




7. Risk analysis

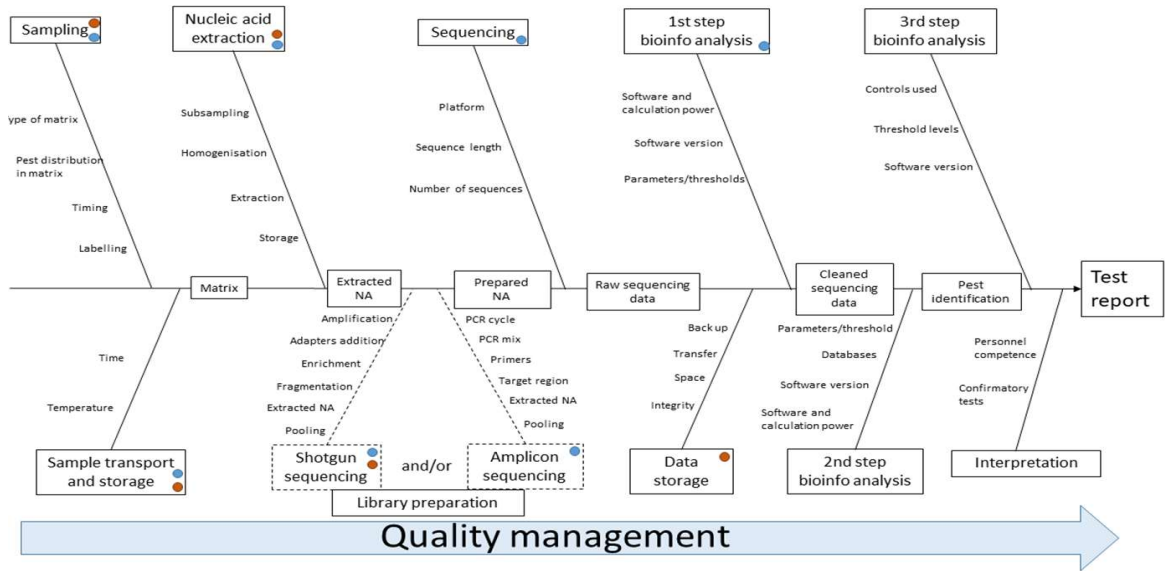


7. Risk analysis





7. Risk analysis



7. Risk analysis

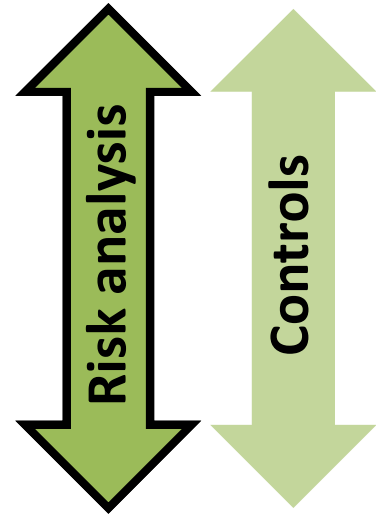


- ▶ What are the risk of HTS testing ?
- ▶ Ishikawa & 6M
- ▶ 28 risks listed, causes and consequences detailed with mitigation measures

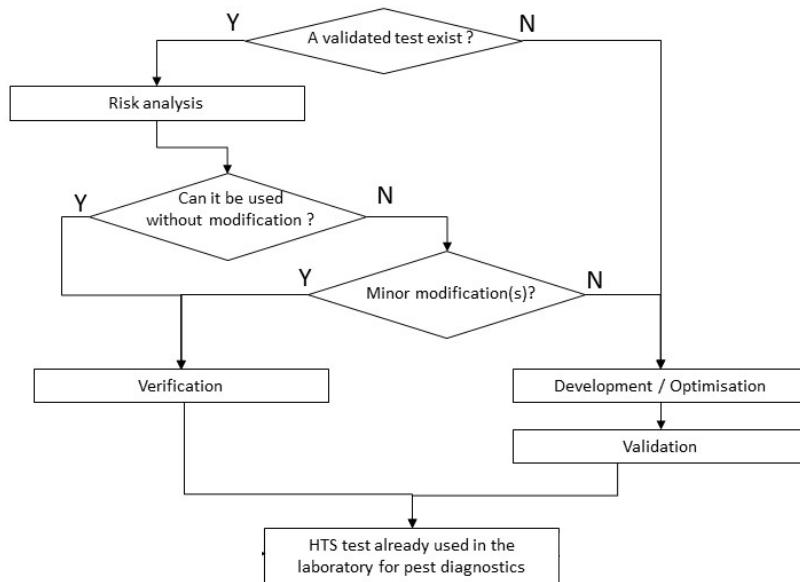


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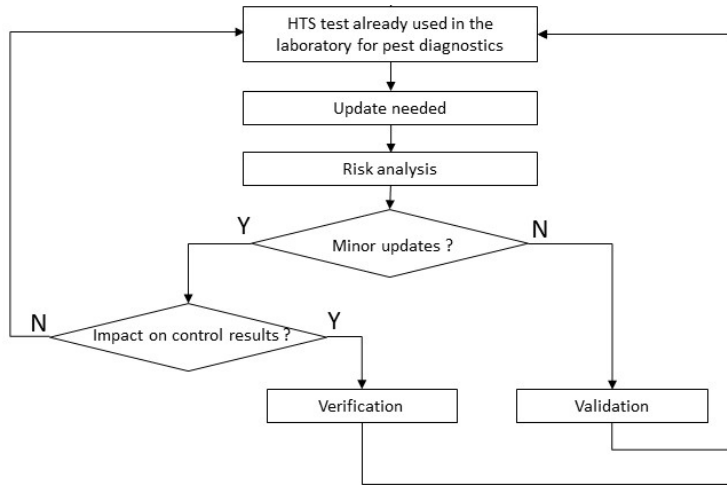


8. How interfacing these steps ?





8. How interfacing these steps ?



Which steps are mandatory for a research lab ?

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Results of the survey



Routine application: quality checks

The control landslide



9. Routine application: controls

► Potential controls listed in the guidelines:

Reference materials	HTS test	Pest range	Notes/remarks
---------------------	----------	------------	---------------



9. Routine application: controls

► Potential controls detailed in the guidelines :

	Negative controls	Positive controls	Alien controls	Internal controls
Aim/Monitoring	Contamination	Contamination Monitor the analytical specificity of the test Monitor the analytical sensitivity of the test when used at low concentration	Contamination Monitor the analytical specificity of the test when use at high concentration Monitor the analytical sensitivity of the test when use at low concentration	Monitor the analytical sensitivity of the test

9. Routine application: controls



► Potential controls detailed in the guidelines

- Description,
- Analysis,
- Steps covered

9. Routine application: controls



► Which control would you use (ressource constrain - efficiciency) ?

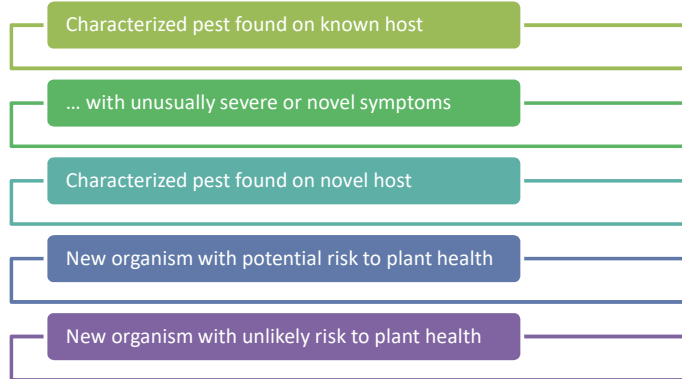
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10. Interpretation of the result

- ▶ Confirmation of identity of the pest
- ▶ Summary table with 5 scenario:



10. Interpretation of the result

- ▶ Confirmation of identity of the pest
- ▶ Summary table with 5 scenario:

Scenario	Confirmatory test required?	Example	Comment
----------	-----------------------------	---------	---------

10. Interpretation of the result



- ▶ Intepretation of the biological relevance
- ▶ Interpretation of the phytosanitary risk
- ▶ For poorly characterized or unkown targets
(are they pests ?)

11. Reporting of the result



- ▶ Inconclusive results ?
- ▶ Unexpected organisms ?
- ▶ Remark and disclaimer ?

Conclusion

Conclusion



- ▶ Comprehensive work to improve the reliability of HTS for diagnostic laboratories & research groups

Conclusion



89 pages in total

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Conclusion



- ▶ Most comprehensive work to improve the reliability of HTS for diagnostic laboratories AND research groups
- ▶ Not linked to any technology (long lasting)
- ▶ WW collaboration of 54 experts

Conclusion



VALITEST reviewers (in alphabetical order)

Adams I., Fox A., Gentit P., Kutjnak D., Mehle N., Petter F., Remenant B., Spadaro D., Trontin C.

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Conclusion



- ▶ Most comprehensive work to improve the reliability of HTS for diagnostic laboratories AND research groups
- ▶ WW collaboration of experts
- ▶ Publicly available soon through 2 scientific publications



Thank you for your attention
& your participation to the pools !

Time for Q&R

Sebastien Massart

sebastien.massart@uliege.be



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



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