Example of method validation based on ISO 17025 and PM 7/98

GEVES
Expertise & Performance

Validation of methods for seedborne pathogens detection

- At GEVES we use official or international (Anses, ISTA, ISHI-Veg) and internal methods for the detection of seedborne pathogens

- We validate the methods following a procedure based on ISO 17025 and PM 7/98

- GEVES through the SHC (V.Grimault) have published a procedure on the ISTA website:

  "Validating methods and organizing and analyzing results of interlaboratory comparative tests (CT)"

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Study of performance criteria

- **To validate a new method or to** compare a new method with an existing one
  - **Aim : validate the p**erformance of the method
    - Analytical sensitivity
    - Diagnostic sensitivity
    - Diagnostic specificity
    - Accuracy
    - Repeatability
    - Reproducibility
    - Robustness

- **For the use of a validated method (e.g. official method)**
  - **Aim: validate the ability of the lab to perform the method**
    - Diagnostic sensitivity
    - Diagnostic specificity
    - Repeatability
    - Reproducibility

- **For a new version of a validated method (e.g. official method)**
  - **Aim: validate the ability of the lab to perform the new version**
    - Identify changes that affect the application of the method
    - Validate the ability of the laboratory to perform the changes

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Validation of methods for seedborne pathogen detection

Performance criteria

- Analytical sensitivity:
  - Limit of detection, smallest amount of the pathogen detected
- Diagnostic sensitivity:
  - ability to detect the target (no false negatives)
- Diagnostic specificity:
  - ability to not detect non target (no false positives)
- Accuracy:
  - combination of diagnostic sensitivity and specificity
- Robustness:
  - ability to not vary according to small variations of parameters in the method
- Repeatability:
  - accord between independant results with same samples, conditions, method
- Reproducibility:
  - accord between independant results with same samples and method in different conditions (analyst, equipment, lab)

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Specificities of seed-borne pathogens detection

- Best to use naturally infected samples at different levels of contamination including healthy (or with saprophytes)
- If not: artificial contamination depending on the kind of pest (viruses, bacteria, fungi, nematodes)
- Quantitative or qualitative results: adapted tools for statistical analysis
- Qualitative: analysis by pools
- Quantitative: (seed by seed)

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Example of a new method version case

- Example of new versions of two methods for the detection of *Xanthomonas* spp. on tomato and pepper seeds.
  - Method for the detection of *Xanthomonas* spp. on tomato seed ISF Version 5
  - Method for the detection of *Xanthomonas* spp. on pepper seed ISF Version 6

- First steps :
  - Determine the impact of the changes and the actions needed
  - Obtain the performance criteria of the validated method (if possible)

Example of a new method version case

Tomato (version 4 -> version 5)	Pepper (version 5 -> version 6)	Actions
Spiking for all seeds, treated and non treated		Already carried out on treated seeds o Modification of the protocol ANA/PAT/ANS/MO/105 o Deletion of the protocol ANA/PAT/ANS/MO/107 o No impact on the staff qualification o No impact on the material o No impact on controls
New recipe of extraction buffer (corresponding to the recipe of extraction buffer for Cmm detection)		o **Validate the ability of the lab to perform the method** o Modification of the protocol ANA/PAT/ANS/MO/105 o Modification of the title of the document ANA/PAT/ANS/E/080 o No impact on the staff qualification o No impact on the material o No impact on controls
Modification of the extraction buffer volume : 4 mL instead 3 mL per g of seeds		o Modification of the protocol ANA/PAT/ANS/MO/105 o No impact on the staff qualification o No impact on the material o No impact on controls
Modification of stomaching duration : Minimum 4 min instead of 7 min		o Modification of the protocol ANA/PAT/ANS/MO/105 o No impact on the staff qualification o No impact on the material o No impact on controls
Modification of the number of colonies to be picked up: 6 instead of 2		Already done in the laboratory
Addition of confirmation of suspect colonies by PCR		o **Validate the ability of the lab to perform the method** o Modification of the protocol ANA/PAT/ANS/MO/105 o Création of the document BIO/ASEQ/E/047 o No impact on the staff qualification o No impact on the material o No impact on controls

Validation ability of the lab to perform the method with the new recipe of the extraction buffer

Example of a new method version case

Performance criteria:

The performance criteria of the validated method were not available.

We decided not to test a dilution near the detection threshold

(Dilutions series could have been closer to the threshold and started at a lower concentration)

- Detection threshold of the new extraction buffer: lower or equivalent than the previous one
  - Check by comparison the ability to recover *Xanthomonas vesicatoria* between the new extraction buffer and the previous one, using a spiking on both media
    - Positive control of *Xanthomonas vesicatoria*
    - Dilution series of each positive control in the new buffer and the previous one

- Repeatability and reproducibility equivalent or higher than the previous one
  - Check by spiking of macerate of healthy seeds
  - 3 repetitions of 3 healthy seed samples for repeatability done at different times for reproductibility
    - Positive control of *Xanthomonas vesicatoria*
    - Macerates of healthy seeds spiked

Example of new method versions case

Results

- Detection threshold of the new extraction buffer lower or equivalent than the previous one

Nb colonies		Previous buffer							New buffer						
Concentration of positive control		10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^1	10^2	10^3	10^4	10^5	10^6	10^7
Xv	CKTM	2	15	80	88	500	TMTC	TMTC	7	30	67	211	529	TMTC	TMTC
	mTMB	260	388	TMTC	TMTC	TMTC	TMTC	TMTC	756	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC
Cfu/mL															
Concentration of positive control		10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^1	10^2	10^3	10^4	10^5	10^6	10^7
Xv	CKTM	20	150	800	880	5000	TMTC	TMTC	70	300	670	2110	5290	TMTC	TMTC
	mTMB	2600	3880	TMTC	TMTC	TMTC	TMTC	TMTC	7560	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC

Counting in number of colonies and Cfu/mL

- The number of colonies was slightly higher with the new extraction buffer

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Example of new method versions case

Results

- Repetability and reproducibility equivalent or higher than the previous one
- The quantitative results of counting were transposed in qualitative results
- Statistical analysis following Langton et al. (2002)

Xv	Previous buffer			New buffer		
Final concentration	10^1	10^2	10^3	10^1	10^2	10^3
Repetability	39%	61%	54%	93%	100%	65%
Reproducibility	11%	36%	33%	78%	100%	75%

% of repetability and reproducibility

- The low percentages were due to one of the 3 samples that had an important saprophytic flora.
- However the percentages were all higher with the new extraction buffer.

Conclusion:

- The performance criteria were reached, the ability of the lab to perform the method with the new recipe of the extraction buffer was validated

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Example of validation of new methods

- GEVES validates also new methods following the procedure based on ISO 17025 and PM 7/98

- Examples of two methods:
  - SE-PCR as prescreening for the detection of *Ditylenchus dipsaci* on Alfalfa seeds
  - Viability test of *Ditylenchus dipsaci*

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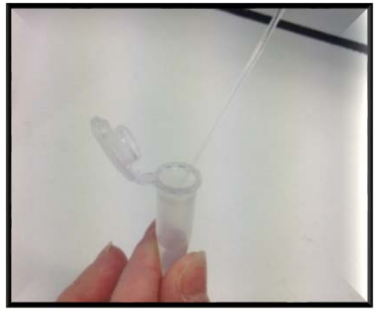
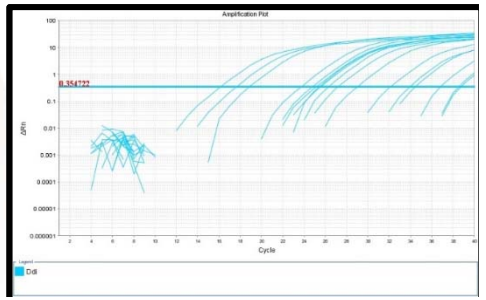
Validation of SE-PCR method

- Analytical sensitivity:
  - Threshold of 0, analytical sensitivity must be 1 *D. dipsaci* in a sample size of 100 grams
- Analytical specificity for primers: done on a collection of different species of *Ditylenchus*
- Diagnostic sensitivity – specificity, accuracy and repeatability:
  - 9 samples: 3 levels of contamination X 3 repetitions
- Reproducibility:
  - 3 levels of contamination X 3 repetitions X 2 labs

Performance criteria results

- Analytical sensitivity: 1 *D. dipsaci*
- Diagnostic sensitivity : 100 %
- Diagnostic specificity: 100 %
- Accuracy : 100 %
- Repeatability : 100 %
- Reproducibility : 100 %





Viability test of *Ditylenchus dipsaci*

- Different ways to kill nematodes = modalities
- Diagnostic sensitivity – specificity, accuracy and repeatability:
  - 4 modalities X 3 repetitions
- Reproducibility:
  - 4 modalities X 3 repetitions X 2 different times

Performance criteria results

- Diagnostic sensitivity : 100 %
- Diagnostic specificity: 100 %
- Accuracy : 100 %
- Repeatability : 100 %
- Reproducibility : 100 %

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