INTER LABORATORY COMPARISON (ILC) REPORT

GEVES
Expertise & Performance

Variety and Seed Study and control Group

National Seed Testing Station - SNES

INTERNATIONAL SEED TESTING ASSOCIATION
UNIFORMITY IN SEED TESTING

2017-ISTA-Bean-BA-Xap, Psp

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Proficiency Test (PT) Report*

Organized by the National Seed Testing Station (SNES) of GEVES for ISTA Seed Health Committee (SHC)

Final

2017-ISTA-Bean-BA-Xap, Psp

Proficiency Test: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Pseudomonas savastanoi* pv. *phaseolicola* in Bean seeds

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Proficiency Test

Detection of *Xanthomonas axonopodis* pv. *phaseoli and Pseudomonas savastanoi* pv. *phaseolicola* in Bean seeds

1 PROFICIENCY TEST ORGANIZATION

The aim of this Proficiency Test was to verify the ability of laboratories to detect *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Pseudomonas savastanoi* pv. *phaseolicola* (Psp) in Bean seeds

Schedule

Sending of samples	13 th of September
Deadline to begin analysis	11 th of October
Deadline to send results	15 th of December
Sending by GEVES of global report and individualized letters	31 th of May

Thirteen laboratories participated in this test and were randomly allocated a number, so that results remained anonymous.

On 13 participants registered for the proficiency test:

-2 of them were accredited for Method 7-023 and 7-021

-11 were not accredited for this method.

2 participants did not receive samples due to problems related to official documents requested and one laboratory did not return results due to experimental problems.

Notation of results

The participants gave a qualitative result (positive, negative) for each sample and for each pathogen and information about the method used.
One participant indicated only the result for *Pseudomonas savastanoi* pv. *phaseolicola.*

Statistical analysis of data

⇨ **Diagnostic sensitivity –specificity**

For homogeneous samples, the analysis was done by addition of the results of the 2 lots (healthy and high level) according to the Standard NF EN ISO 16140 which expresses results as presence/absence.

This norm gives us performance assessment criteria on diagnostic sensitivity, diagnostic specificity and accuracy calculated as follows:

	expected result + (contaminated sample)	expected result - (healthy sample)
Obtained result +	positive agreement +/+ (PA)	positive deviation -/+ (PD)
Obtained result -	negative deviation +/- (ND)	negative agreement -/- (NA)

Sensitivity: Percentage of samples correctly identified as positives. ΣPA/(ΣPA+ΣND)x100.
Specificity: Percentage of samples correctly identified as being negative. ΣNA/(ΣNA+ΣPD)× 100.
Accuracy: (ΣNA+ΣPA)/ (ΣPA+ΣNA+ΣPD+ΣND) x100.

PA = positive agreement
ND = negative deviation
NA = negative agreement
PD = positive deviation
N = total number of possible agreement

Conformity of results:

Performance criteria	Level to obtain
Sensitivity	100%: all contaminated samples are positive; no false negative results have been obtained
Specificity	100%: all healthy samples are negative; no false positive results have been obtained
Accuracy	Synthesis of the two performance criteria. So, no false positive or negative results have been obtained

The analysis of the results for a participating laboratory led to a declaration of conformity or non-conformity of the results in an individual sheet.
- “conform”: obtained results correspond to expected results.
- “not conform”: obtained results do not correspond to expected results.

⇨ **Seedcalc8 and Probability ISTA tools:**

Seedcalc8:

Seedcalc program is a “probability tool for qualitative results” provided on the STATCOM webpage (tools), used to determine the % of contamination of the seed.

⇨ **Probability of k positive samples out of n :**

Probability tool is provided on the SHC webpage (tools) and used to calculate the probability to find a number of contaminated samples over the number tested from the % determined with Seedcalc8 tool.

⇨ **Rating system**

(For information, only)

The rating system is under development and these results are given for information only.

The calculation of the rating is done with the Excel file developed in collaboration with the Statistical committee of ISTA. It is based on an A, B, C and BMP rating.

In this case:

- A corresponds to an expected result using a probability of 5%, and no false positive in healthy level.
- B using a probability of 2.5%, and 1 false positive in healthy level.
- C using a probability under 1%, and 1 false positive in healthy level.
- BMP (Below Minimum Performance) corresponds to a not expected result. A false positive in healthy level or false negative in high level lead to BMP.

Validation of samples

The samples have been validated through homogeneity and stability tests.

The results of participating laboratories were compared to the expected results determined by the homogeneity test which results were confirmed by the stability test.

Pretest

Five lots naturally contaminated with different levels (healthy, medium and high levels) have been tested in five subsamples of 1000 seeds by ISTA method the 19 th of January 2017. The characteristics of the five lots are shown in table n°1 and the results of the pretest in table n°2.

Table n°1: Characteristic of the lots

Codification	Color	TSW (thousand seed weight) in grams	Pathogens		Healthy
			Xap	Psp	
A	red	620	X	X	
B	white	642	X		
C	white	389			X
D	red	479			X
E	white	173		X	

Different colors and different TSW have been found.

Table n°2: Results of pretest

Codification	Results of Pathogens			
	Xap		**Psp**	
	Level of contamination	Results	Level of contamination	Results
A	**M**edium	$1^+/5$	**M**edium	$3^+/5$
B	**M**edium	$3^+/5$	Healthy	$0^+/5$
C	**H**ealthy	$0^+/5$	Healthy	$0^+/5$
D	**H**ealthy	$0^+/5$	Healthy	$0^+/5$
E	**H**ealthy	$0^+/5$	**Hi**gh	$5^+/5$

One lot (lot A) was contaminated with both pathogens and two lots were contaminated with 1 out of 2 different pathogens.

For *Pseudomonas savastanoi* pv. *phaseolicola* (Psp) , we had 2 levels of contamination (medium and high).

For *Xanthomonas axonopodis* pv. *phaseoli* (Xap), we obtained the medium level.

The healthy lots were negative results.

Homogeneity Test

Homogeneity test was done after packaging and just before sending. 10 extra samples of 1 000 seeds representing each contamination level were tested. The results are given in Table n°3.

The samples have been tested the 17 th of July 2017.

Table n°3: Results of homogeneity test.

Codification	Level of contamination (based on pretest)	Results of homogeneity test	
		Xap	Psp
A	Medium (Xap ; Psp)	0+/10	8+/10
B	Medium (Xap)	8+/10	0+/10
C	Healthy	0+/10	0+/10
D	Healthy	0+/10	0+/10
E	High (Psp)	0+/10	9+/10

- **Results for healthy samples**
  All samples were negative as expected
- **Results for samples with medium level of contamination**
  For Xap
  Lot A, the obtained result was 0 positives samples out of 10 tested. This result was lower than the pretest (1 out of 5 positives samples). These variations could occur for a naturally contaminated seed lot.
  Lot B, the obtained result was 8 positives samples out of 10, this result was higher than those of pretest.

  For Psp
  Lot A, the obtained result was 8 positives samples out of 10, this result was higher than those of pretest.
- **Results for samples with high level of contamination**
  For Psp
  The obtained result was 9 positive samples out of 10 tested whereas the expected result was 10 positive samples out of 10 tested.
- **Conclusion of homogeneity test**

- For healthy level, we obtained 0 positive samples on 2 lots. No false positive obtained.

- For medium level:

Psp : the result obtained is in accordance with the expected results.

Xap: the result obtained is lower than expected, the lot is slightly contaminated, which explains this variability.

- For the high level:

Psp : the result obtained is lower than expected, the contamination of the lot started to decrease . The high level is therefore reclassified as medium level.

We decided to keep the 5 tested lots.

Seed samples

21 samples of 1 000 bean seeds have been sent to each participant with different number of replicates depending on the level of contamination (table n°4).

Table n°4: Characteristics of samples

Codification	Number of samples	Level of contamination			Expected value
		Xap	Psp	Healthy	
A	5	X	X		Positive both pathogens
B	5	X			Positive Xap
C	3			X	Negative
D	3			X	Negative
E	5		X		Positive Psp

Each sample was sent in a sealed bag.

Stability Test

The stability test was done after the last confirmation of starting of analysis by laboratories. The stability test has been started the 13th of February 2018.

Considering the variability observed during the pretests and homogeneity test we decided, in order to obtain a relevant statistical analysis to increase the number of samples analyzed for contaminated lots.

Codification of lot	Number of samples
A	10
B	10
C	3
D	3
E	10

3 extra samples of 1 000 seeds were tested for each healthy lot and 10 extra samples of 1000 seeds for the other levels.

Results are given in Table n°5.

Table n°5: Results of stability test.

lot	Level of contamination	Pathogens	Xap		Psp	
			homogeneity	stability	homogeneity	stability
A	Medium	Xap + Psp	$0^{+}/10$	$3^{+}/10$	**$8^{+}/10$**	$7^{+}/10$
B	Medium	Xap + Psp	**$8^{+}/10$**	$10^{+}/10$	$0^{+}/10$	$3^{+}/10$
C	Healthy		$0^{+}/10$	$0^{+}/3$	$0^{+}/10$	$0^{+}/3$
D	Healthy		$0^{+}/10$	$0^{+}/3$	$0^{+}/10$	$0^{+}/3$
E	Medium	Psp	$0^{+}/10$	$0^{+}/10$	**$9^{+}/10$**	$7^{+}/10$

⇨ **Results of healthy lot**
There were no positive samples.

⇨ **Results of medium level**
Result of homogeneity test was used for the computation of probability to obtain contaminated samples out of tested samples. The percentage of infection obtained with homogeneity test (upper bound at 95% confidence) allowed an extrapolation to know the number of positive samples expected for the stability test (figure 1 to 4).

For $8^{+}/10$

Figure n°1: Results of medium level with the Seedcalc8 software

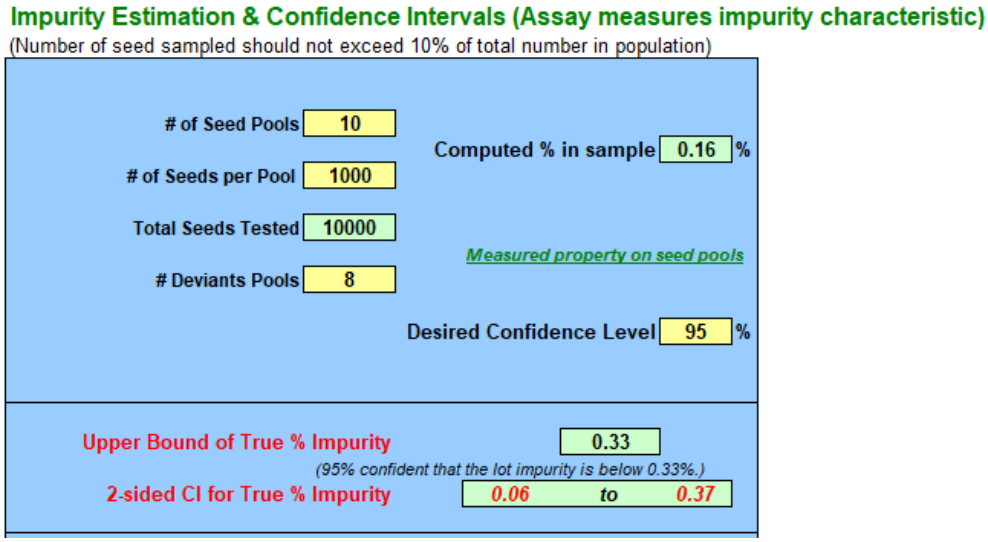
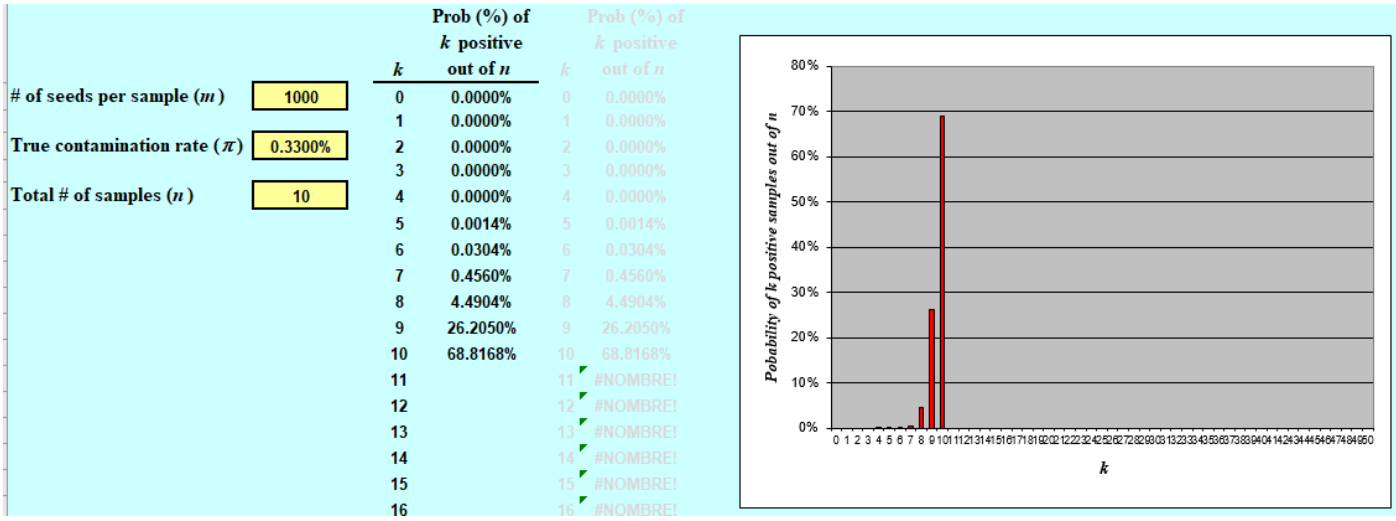


Figure n°2: Expected number of contaminated samples according to infection rate.

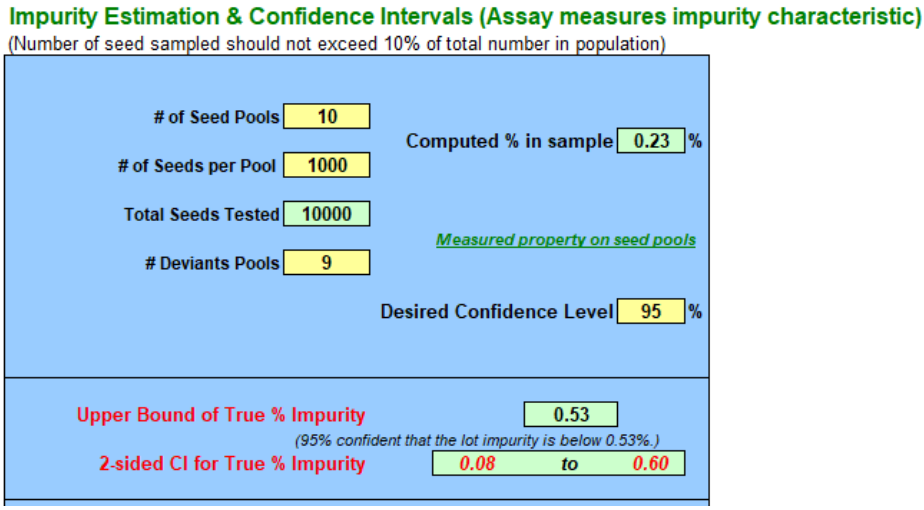


# of seeds per sample (m)	1000
True contamination rate (π)	0.3300%
Total # of samples (n)	10

k	Prob (%) of k positive out of n
0	0.0000%
1	0.0000%
2	0.0000%
3	0.0000%
4	0.0000%
5	0.0014%
6	0.0304%
7	0.4560%
8	4.4904%
9	26.2050%
10	68.8168%
11	
12	
13	
14	
15	
16	

For $9^{+}/10$

Figure n°3: Results of medium level with the Seedcalc8 software

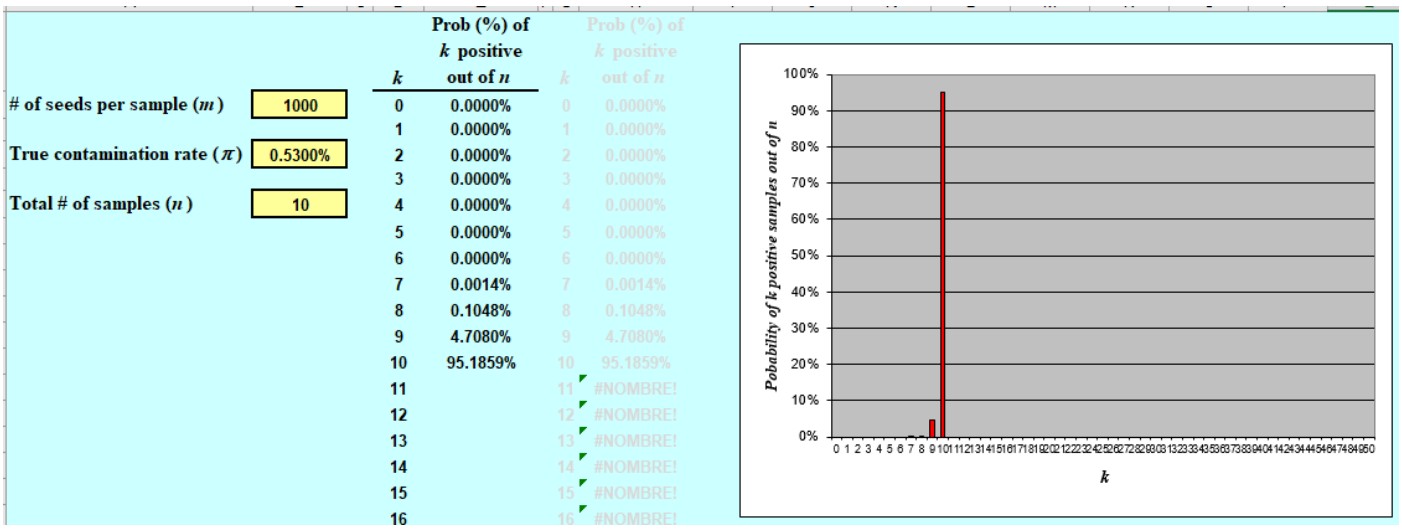


Impurity Estimation & Confidence Intervals (Assay measures impurity characteristic)

(Number of seed sampled should not exceed 10% of total number in population)

# of Seed Pools	10
# of Seeds per Pool	1000
Total Seeds Tested	10000
# Deviants Pools	9
Computed % in sample	0.23 %
Measured property on seed pools	
Desired Confidence Level	95 %
Upper Bound of True % Impurity	0.53
(95% confident that the lot impurity is below 0.53%.)	
2-sided CI for True % Impurity	*0.08* to *0.60*

Figure n°4: Expected number of contaminated samples according to infection rate.



# of seeds per sample (m)	1000
True contamination rate (π)	0.5300%
Total # of samples (n)	10

k	Prob (%) of k positive out of n
0	0.0000%
1	0.0000%
2	0.0000%
3	0.0000%
4	0.0000%
5	0.0000%
6	0.0000%
7	0.0014%
8	0.1048%
9	4.7080%
10	95.1859%
11	
12	
13	
14	
15	
16	

⇨ **Conclusion of the stability test**

The table n°6 is a summary to expected and obtained results.

Table n°6: Summary to expected/obtained results

Lot	Pathogen	Homogeneity results	Stability test		Result
			Expected number of contaminated	Obtained number of contaminated	In or out expected value
A	Psp	$8^{+}/10$	9 to $10^{+}/10$	$7^{+}/10$	Out
A	Xap	$0^{+}/10$		$3^{+}/10$	
B	Xap	$8^{+}/10$	9 to $10^{+}/10$	$10^{+}/10$	In
B	Psp	$0^{+}/10$		$3^{+}/10$	
E	Psp	$9^{+}/10$	$10^{+}/10$	$9^{+}/10$	Out

Stability of the lots has been confirmed:

- For the healthy lot, we obtained 0 positive samples which was the same result than homogeneity test. No false positive obtained.

- For the medium level:

For Psp: result obtained is lower than the expected result, showing a slight decrease of contamination for lots A and E. This pathogen was also detected in this test for lot B, it has been taken into account in the statistical analysis

For Xap: we obtained 10 positive samples out of 10, which correspond to the probability.

The stability test confirmed the presence of Xap in lot A, detected in the pretest and not in the homogeneity test

2 PROFICIENCY TEST RESULTS

Raw data of all laboratories are given in appendix.
A summary table of the results found by batch with all participants is presented below:

Table n°7: data by lot for all participants

Lot	N° lab	*Xanthomonas* (Xap)	*Pseudomonas* (Psp)
A	01	$0^+/5$	$2^+/5$
	02	$1^+/5$	$4^+/5$
	03	$1^+/5$	$5^+/5$
	05	$0^+/5$	$3^+/5$
	06	$0^+/5$	$3^+/5$
	07	$1^+/5$	$2^+/5$
	08	not realized	$3^+/5$
	09	$1^+/5$	$3^+/5$
	10	$1^+/5$	$4^+/5$
	12	$1^+/5$	$3^+/5$
B	01	$5^+/5$	$0^+/5$
	02	$5^+/5$	**$3^+/5$**
	03	$5^+/5$	$0^+/5$
	05	$4^+/5$	$0^+/5$
	06	$5^+/5$	$0^+/5$
	07	$3^+/5$	$0^+/5$
	08	not realized	$0^+/5$
	09	$4^+/5$	**$1^+/5$**
	10	$5^+/5$	$0^+/5$
	12	$5^+/5$	$0^+/3$ +2 und
C	01	$0^+/3$	$0^+/3$
	02	**$1^+/3$**	**$1^+/3$**
	03	$0^+/3$	$0^+/3$
	05	$0^+/3$	$0^+/3$
	06	$0^+/3$	$0^+/3$
	07	$0^+/3$	$0^+/3$
	08	not realized	$0^+/3$
	09	$0^+/3$	$0^+/3$
	10	$0^+/3$	$0^+/3$
	12	$0^+/3$	$0^+/3$
D	01	$0^+/3$	$0^+/3$
	02	$0^+/3$	$0^+/3$
	03	**$1^+/3$**	$0^+/3$
	05	$0^+/3$	$0^+/3$
	06	$0^+/3$	$0^+/3$
	07	$0^+/3$	$0^+/3$
	08	not realized	$0^+/3$
	09	$0^+/3$	$0^+/3$
	10	$0^+/3$	$0^+/3$
	12	$0^+/3$	$0^+/3$
E	01	$0^+/5$	$5^+/5$
	02	$0^+/5$	$4^+/5$
	03	$0^+/5$	$5^+/5$
	05	$0^+/5$	$5^+/5$
	06	$0^+/5$	$4^+/5$
	07	$0^+/5$	$4^+/5$
	08	not realized	$4^+/5$
	09	$0^+/5$	$5^+/5$
	10	**$1^+/4$** + 1 und	$5^+/5$
	12	$0^+/1$ + 4 und	$4^+/4$ +1 und

und = undetermined

All results found inconclusive or undetermined by the labs were not used for the statistical analysis and were excluded, they were given a B ranking for the corresponding samples as a result was expected and it was possible to analyze the samples. For statistical analysis it is better to have a positive or negative result. The next result file will be modified in this way.

Results of healthy level

Analysis of results of healthy level has been carried out according to the Norm NF EN ISO 16140 suitable to results expressed as positive / negative.
Results are given in table n°8 and n°9.

Table n°8: Expected results

Lot	Nb of samples	Xap	Psp
A	5	X	X
B	5	X	X
C	3	0	0
D	3	0	0
E	5	0	X

Table n°9: Analysis of qualitative results for each laboratory for healthy level.

Lab number	**Healthy**	
	Xap	Psp
01	$0^{+}/11$	$0^{+}/6$
02	$1^{+}/11$	$1^{+}/6$
03	$1^{+}/11$	$0^{+}/6$
05	$0^{+}/11$	$0^{+}/6$
06	$0^{+}/11$	$0^{+}/6$
07	$0^{+}/11$	$0^{+}/6$
08		$0^{+}/6$
09	$0^{+}/11$	$0^{+}/6$
10	$1^{+}/11$	$0^{+}/6$
12	$0^{+}/11$	$0^{+}/6$

- detection not realized

Criteria of performance as specificity per lab are indicated in Table n°10.

Table n°10: Criteria of performance for healthy level for each laboratory

Lab number	Specificity %	
	Xap	Psp
01	100.00	100.00
02	91.67	85.71
03	91.67	100.00
05	100.00	100.00
06	100.00	100.00
07	100.00	100.00
08		100.00
09	100.00	100.00
10	91.67	100.00
12	100.00	100.00

Evaluation of performance criteria of participants:

- Xap: Six laboratories obtained 100% of specificity (no false positive) and three laboratories obtained 1 out of 11 positive results.
  - Lab 02 obtained 1 positive result in lot C
  - Lab 03 obtained 1 positive result in lot D
  - Lab 10 obtained 1 positive result in lot E

- Psp: Nine laboratories obtained 100% of specificity (no false positive) and one laboratory obtained positive results (Lab 02).
  - Lab 02 obtained 1 positive result in lot C

Results for medium level

Considering the variability observed during the tests we decided, in order to obtain a relevant statistical analysis to use all results, homogeneity and stability tests.
The statistical analysis will be based on the result in table n°11.

Table n°11: Value to calculate the expected number of positive samples

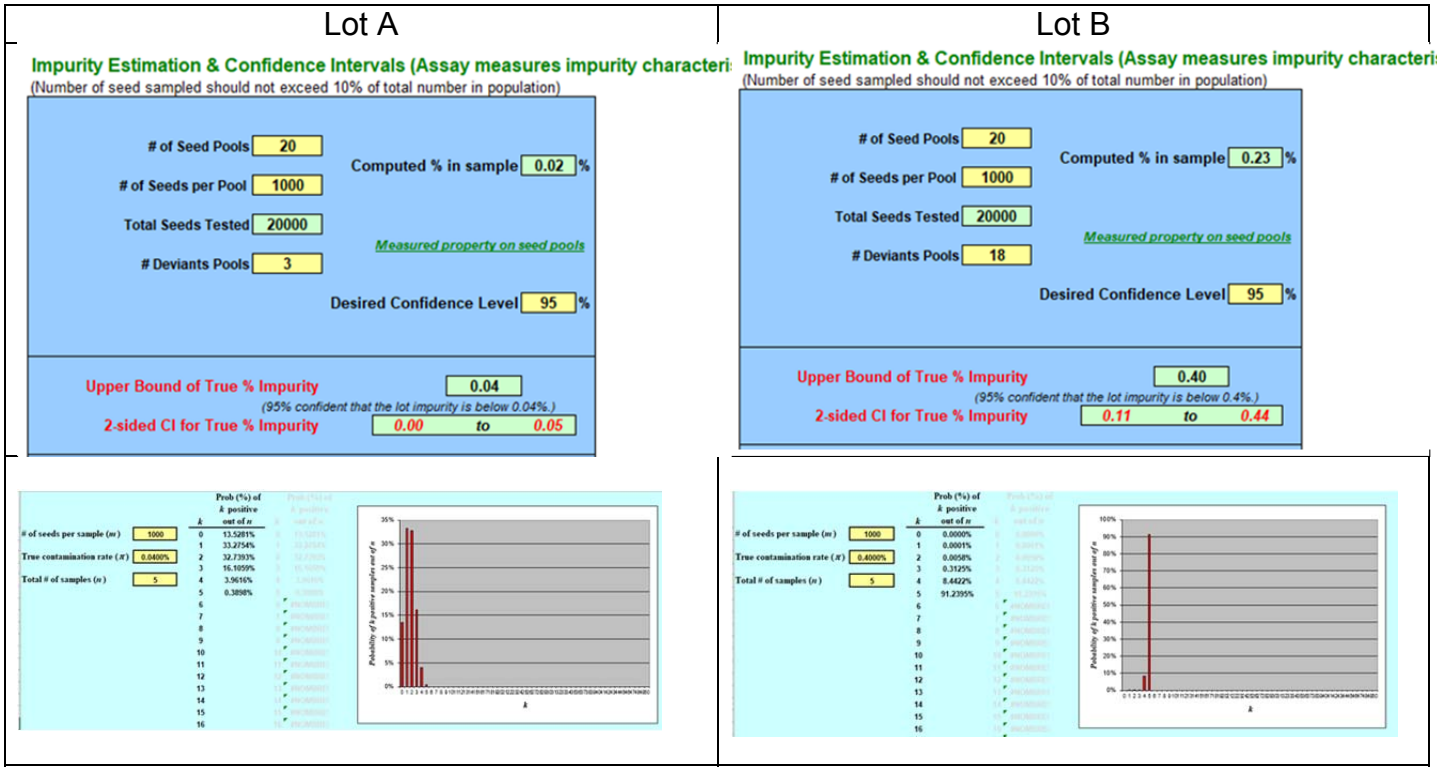
lot	Xap	Psp
A	$3^{+}/20$	$15^{+}/20$
B	$18^{+}/20$	$3^{+}/20$
E		$16^{+}/20$

Result of test was used for the computation of probability to obtain contaminated samples out of tested samples. The percentage of infection obtained was:

Xap (figure n°5):

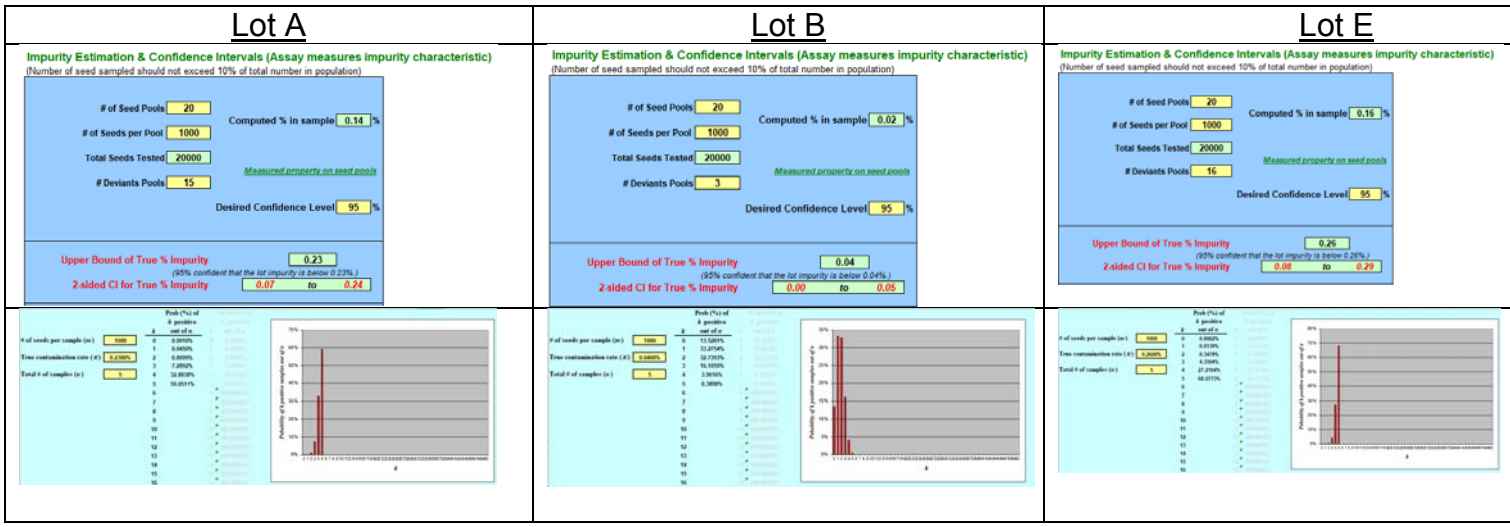
Lot A : 0.04% (upper bound at 95% confidence), corresponding to 0 to 3 positive samples out of 5.
Lot B : 0.40% (upper bound at 95% confidence), corresponding to 4 to 5 positive samples out of 5.

Figure n°5: Results of Xap medium levels with the Seedcalc8 software.



Psp (figure n°6):
Lot A : 0.23% (upper bound at 95% confidence), corresponding to 3 to 5 positive samples out of 5.
Lot B: 0.04% (upper bound at 95% confidence), corresponding to 0 to 3 positive samples out of 5.
Lot E : 0.26% (upper bound at 95% confidence), corresponding to 4 to 5 positive samples out of 5.

Figure n°6: Results of Psp medium level with the Seedcalc8 software



Results for each laboratory are given in table n°12.

Table n° 12: analysis of results of laboratories for medium level

N° lab	lot	Xap		Psp	
		expected	obtained	expected	obtained
01	A	0-3	0	3-5	**2**
	B	4-5	5	0-3	0
	E			4-5	5
02	A	0-3	1	3-5	4
	B	4-5	5	0-3	3
	E			4-5	4
03	A	0-3	1	3-5	5
	B	4-5	5	0-3	0
	E			4-5	5
05	A	0-3	0	3-5	3
	B	4-5	4	0-3	0
	E			4-5	5
06	A	0-3	0	3-5	3
	B	4-5	5	0-3	0
	E			4-5	4
07	A	0-3	1	3-5	**2**
	B	4-5	**3**	0-3	0
	E			4-5	**2**
08	A			3-5	3
	B			0-3	0
	E			4-5	4
09	A	0-3	1	3-5	3
	B	4-5	4	0-3	1
	E			4-5	5
10	A	0-3	1	3-5	4
	B	4-5	5	0-3	0
	E			4-5	5
12	A	0-3	1	3-5	3
	B	4-5	5	0-3	0
	E			4-5	4

cell in grey correspond to laboratories' results different from expected ones

For Xap: Eight participants obtained the expected value range (from 0 to 4 and 4 to 5) and one participant obtained a result under the limit (Lab 07).
For Psp: Eight participants obtained the expected value range, two participants obtained a result under the limit (Lab 01 and lab 07).

Z-score-computations and rating system rating system (for information)

We proposed to do a rating for each pathogen.

a) Xap

Rating is based on the number of positive and negative samples. The two lots A and B have very different levels of contamination. If we had combined the rates we would have made a deviation. Because of the current parameters of the software, it was decided to cancel the “high level” for the statistical analysis and to duplicate the “medium level” to obtain a rating with the 2 levels.

For the healthy samples, the expected result was 0 positive samples to obtain letter A and we decided to accept a deviation with 1 positive to be rated as B.

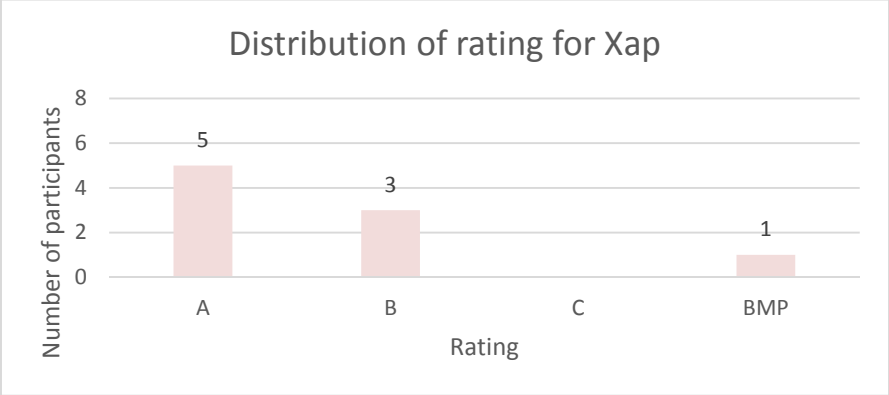
For the medium level sample, we used the rate determined by the probability tool. Results with a probability <5% were rated B (>2.5%) or C (> 1%) or BMP (<1%) for each lot.

The results are presented in table n°13 and distribution of rating figure n°7

Table n°13: Computations of laboratories and rating.

Rating for qualitative SH PTs				Change any value in a yellow cell					
	Minimum requirements for A rating :				Lot B			Lot A	
		Healthy lot			Medium level lot			Medium level lot	
	Max # of pos reps:	0	and	Min # of pos reps:	5.0%	and	Min prob for observing k pos:	5.0%	
	Minimum requirements for B rating :								
		Healthy lot			Medium level lot			Medium level lot	
	Max # of pos reps:	1	and	Min # of pos reps:	2.5%	and	Min prob for observing k pos:	2.5%	
	Minimum requirements for C rating :								
		Healthy lot			Medium level lot			Medium level lot	
	Max # of pos reps:	1	and	Min # of pos reps:	1.0%	and	Min prob for observing k pos:	1.0%	
					Medium level lot			Medium level lot	
		Healthy lot		# of seeds/rep:	1000		# of seeds/rep:	1000	
				True cont. rate:	0.40%		True cont. rate:	0.0400%	
				# of reps tested:	5		# of reps tested:	5	
Rating	Lab	# of pos reps			# of pos reps	Prob for observing k pos		# of pos reps (k)	Prob for observing k pos
A	1	0			5	91.24%		0	13.53%
B	2	1			5	91.24%		1	33.28%
B	3	1			5	91.24%		1	33.28%
A	5	0			4	8.44%		0	13.53%
A	6	0			5	91.24%		0	13.53%
BMP	7	0			3	0.31%		1	33.28%
A	9	0			4	8.44%		1	33.28%
B	10	1			5	91.24%		1	33.28%
A	12	0			5	91.24%		1	33.28%

Figure n°7: Distribution of rating



At the final, five participants achieved an A rating, three participants achieved a B rating and one achieved BMP.
The BMP rating is due a value below the expected threshold in the lot B for Lab 07.

b) Psp

Rating is based on the number of positive and negative samples. The two lots A and B have the same levels of contamination. Because of the current parameters of the software, it was decided to cancel the “high level” for the statistical analysis and to duplicate the “medium level” to obtain a rating with the 2 levels.

For the healthy samples, the expected result was 0 positive samples to obtain letter A and we decided to accept a deviation with 1 false positive. to be rated as B.

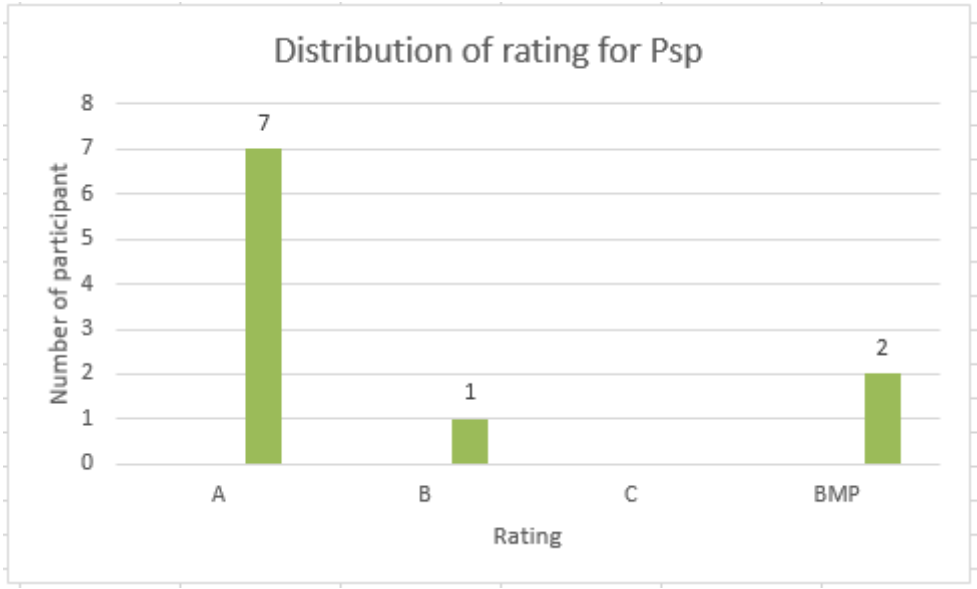
For the medium level sample, we used the rate determined by the probability tool. Results with a probability <5% were rated B (>2.5%) or C (> 1%) or BMP (<1%) for each lot.

The results are presented in table n°14 and distribution of rating in figure n°8.

Table n°14: Computations of laboratories and rating.

Rating for qualitative SH PTs				Change any value in a yellow cell								
Minimum requirements for A rating :					Lot E			Lot A			Lot B	
		Healthy lot			Medium level lot			Medium level lot			Medium level lot	
	Max # of pos reps:	0	and	Min # of pos reps:	5.0%	and	Min prob for observing k pos:	5.0%		Min prob for observing k pos:	5.0%	
Minimum requirements for B rating :												
		Healthy lot			Medium level lot			Medium level lot			Medium level lot	
	Max # of pos reps:	1	and	Min # of pos reps:	2.5%	and	Min prob for observing k pos:	2.5%		Min prob for observing k pos:	2.5%	
Minimum requirements for C rating :												
		Healthy lot			Medium level lot			Medium level lot			Medium level lot	
	Max # of pos reps:	1	and	Min # of pos reps:	1.0%	and	Min prob for observing k pos:	1.0%		Min prob for observing k pos:	1.0%	
					Medium level lot			Medium level lot			Medium level lot	
		Healthy lot		# of seeds/rep:	1000		# of seeds/rep:	1000			1000	
				True cont. rate:	0.26%		True cont. rate:	0.23%			0.04%	
				# of reps tested:	5		# of reps tested:	5			5	
Rating	Lab	# of pos reps			# of pos reps	Prob for observing k pos		# of pos reps (k)	Prob for observing k pos		# of pos reps (k)	Prob for observing k pos
BMP	1	0			5	68.08%		2	0.81%		0	13.53%
B	2	1			4	27.21%		4	32.80%		3	16.11%
A	3	0			5	68.08%		5	59.05%		0	13.53%
A	5	0			5	68.08%		3	7.29%		0	13.53%
A	6	0			4	27.21%		3	7.29%		0	13.53%
BMP	7	0			2	0.35%		2	0.81%		0	13.53%
A	8	0			4	27.21%		3	7.29%		0	13.53%
A	9	0			5	68.08%		3	7.29%		1	33.28%
A	10	0			5	68.08%		4	32.80%		0	13.53%
A	12	0			4	27.21%		3	7.29%		0	13.53%

Figure n°8: Distribution of rating

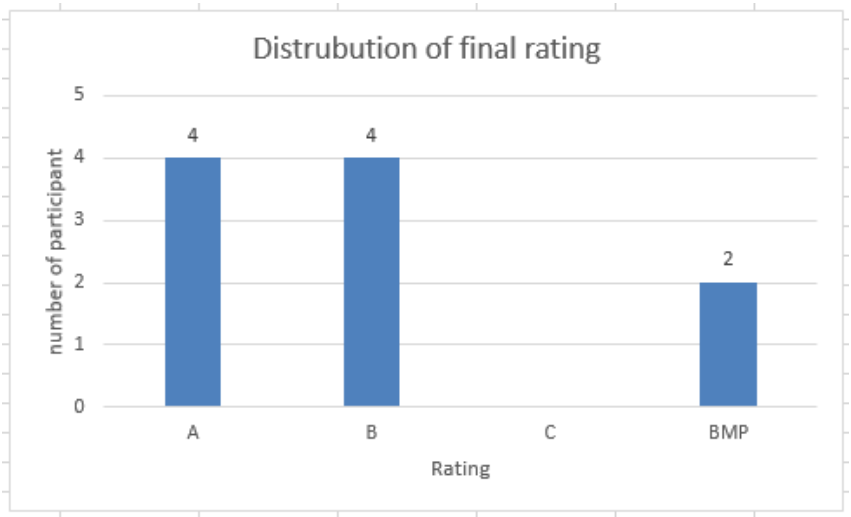


At the final, seven participants achieved an A rating and the one participants achieved a B rating and two achieved a BMP rating.
The BMP rating is due to a value below the expected threshold in lot A for lab 1 and in lot A and E for lab 7.

-Final Rating
The final rating represents the obtained letter in one of different ratings.

Lab number	Qualitative		Undetermined results	Summary of rating
	Xap	Psp		
01	A	BMP	/	BMP
02	B	B	/	B
03	B	A	/	B
05	A	A	/	A
06	A	A	/	A
07	BMP	BMP	/	BMP
08		A	/	A
09	A	A	/	A
10	B	A	B	B
12	A	A	B	B

Figure n°9: Distribution of final rating



The sum of letter A; B represent 80% of ratings.
2 participants achieved a BMP, it is due to a value below the expected threshold.

3 Conclusion

For this proficiency test, half of the participants obtained an A rating and if we add the letter B we obtain a rate of 80%.

Lot A showed results lower than expected for 2 participants for the detection of Psp. This lot was very dirty, containing soil and vegetal fragments. It can explain the variability of the results.

Lot B showed intralot variability during the test process due to the low level of contamination.

Lots C and D were confirmed healthy during the test process. 2 participants obtained a positive result, one for lot C and one for lot D.

Lot E come from a 'home' production carried out by contamination by Psp of the plant in a plot of GEVES. This lot was high for Psp in the pretest. The contamination decreased but not strongly. The presence of saprophyte may have complicated the notation.

All participants used a method to verify the suspect colonies of pathogens, below the technics used.

Lab number	Xap		Psp	
	PCR	Pathogenicity test	PCR	Pathogenicity test
01	X			X
02	X	X		X
03	X		X	X
05	X	X		X
06	X			X
07	X		X	
08			X	X
09	X			X
10	X	X	X	X
12	X		X	

There is no correlation between the technique used and the results obtained by participants.

The low contamination of some lots led to a high variability in the results between the pretests, the homogeneity test and the stability test. This led us to increase the number of replicates for statistical analysis. In the case of analyses with several pathogens with a large number of seeds per sample, and because of the quarantine status of Xap, it has been difficult to obtain high contaminated lots and to have a number of replicates allowing an easier statistical analysis.

APPENDIX

Table 1: Raw data:

			Xanthomonas (Xap)		Pseudomonas (Psp)	
Lab number	Codification of lot	Number of samples	Obtained results	Expected results	Obtained results	Expected results
01	A	11	-	+/-	+	+/-
		36	-	+/-	-	+/-
		140	-	+/-	+	+/-
		210	-	+/-	-	+/-
		266	-	+/-	-	+/-
	B	13	+	+/-	-	+/-
		31	+	+/-	-	+/-
		37	+	+/-	-	+/-
		86	+	+/-	-	+/-
		226	+	+/-	-	+/-
	C	2	-	-	-	-
		131	-	-	-	-
		171	-	-	-	-
	D	12	-	-	-	-
		33	-	-	-	-
		261	-	-	-	-
	E	19	-	-	+	+/-
		65	-	-	+	+/-
		68	-	-	+	+/-
		176	-	-	+	+/-
		233	-	-	+	+/-
02	A	1	-	+/-	-	+/-
		118	-	+/-	+	+/-
		145	-	+/-	+	+/-
		164	+	+/-	+	+/-
		229	-	+/-	+	+/-
	B	7	+	+/-	+	+/-
		80	+	+/-	+	+/-
		134	+	+/-	+	+/-
		165	+	+/-	-	+/-
		244	+	+/-	-	+/-
	C	79	-	-	-	-
		117	-	-	-	-
		137	+	-	+	-
	D	222	-	-	-	-
		249	-	-	-	-
		262	-	-	-	-
	E	52	-	-	+	+/-
		113	-	-	+	+/-
		199	-	-	+	+/-
		205	-	-	+	+/-
		208	-	-	-	+/-
03	A	54	-	+/-	+	+/-
		115	-	+/-	+	+/-
		187	+	+/-	+	+/-
		227	-	+/-	+	+/-
		230	-	+/-	+	+/-
	B	46	+	+/-	-	+/-
		99	+	+/-	-	+/-
		129	+	+/-	-	+/-
		138	+	+/-	-	+/-
		197	+	+/-	-	+/-
	C	104	-	-	-	-
		112	-	-	-	-
		136	-	-	-	-
	D	49	+	-	-	-
		191	-	-	-	-
		192	-	-	-	-
	E	50	-	-	+	+/-
		163	-	-	+	+/-
		167	-	-	+	+/-
		202	-	-	+	+/-
		252	-	-	+	+/-

			Xanthomonas (Xap)		Pseudomonas (Psp)	
Lab number	Codification of lot	Number of samples	Obtained results	Expected results	Obtained results	Expected results
05	A	9	-	+/-	+	+/-
		47	-	+/-	+	+/-
		181	-	+/-	-	+/-
		250	-	+/-	+	+/-
		273	-	+/-	-	+/-
	B	64	+	+/-	-	+/-
		107	+	+/-	-	+/-
		152	+	+/-	-	+/-
		193	+	+/-	-	+/-
		215	-	+/-	-	+/-
	C	30	-	-	-	-
		267	-	-	-	-
		271	-	-	-	-
	D	16	-	-	-	-
		162	-	-	-	-
		198	-	-	-	-
	E	61	-	-	+	+/-
		62	-	-	+	+/-
		158	-	-	+	+/-
		209	-	-	+	+/-
		213	-	-	+	+/-
06	A	8	-	+/-	-	+/-
		73	-	+/-	+	+/-
		83	-	+/-	+	+/-
		259	-	+/-	-	+/-
		268	-	+/-	+	+/-
	B	3	+	+/-	-	+/-
		82	+	+/-	-	+/-
		173	+	+/-	-	+/-
		174	+	+/-	-	+/-
		184	+	+/-	-	+/-
	C	178	-	-	-	-
		143	-	-	-	-
		189	-	-	-	-
	D	17	-	-	-	-
		32	-	-	-	-
		151	-	-	-	-
	E	66	-	-	+	+/-
		146	-	-	+	+/-
		223	-	-	+	+/-
		246	-	-	+	+/-
		272	-	-	-	+/-
07	A	5	-	+/-	-	+/-
		26	-	+/-	+	+/-
		59	+	+/-	+	+/-
		200	-	+/-	-	+/-
		263	-	+/-	-	+/-
	B	122	-	+/-	-	+/-
		155	-	+/-	-	+/-
		166	+	+/-	-	+/-
		221	+	+/-	-	+/-
		228	+	+/-	-	+/-
	C	139	-	-	-	-
		170	-	-	-	-
		195	-	-	-	-
	D	206	-	-	-	-
		216	-	-	-	-
		235	-	-	-	-
	E	75	-	-	-	+/-
		89	-	-	-	+/-
		95	-	-	+	+/-
		144	-	-	+	+/-
		172	-	-	-	+/-

			Xanthomonas (Xap)		Pseudomonas (Psp)	
Lab number	Codification of lot	Number of samples	Obtained results	Expected results	Obtained results	Expected results
08	A	239	not realized	+/-	+	+/-
		177		+/-	+	+/-
		148		+/-	+	+/-
		23		+/-	-	+/-
		257		+/-	-	+/-
	B	168	not realized	+/-	-	+/-
		255		+/-	-	+/-
		219		+/-	-	+/-
		60		+/-	-	+/-
		178		+/-	-	+/-
	C	256	not realized	-	-	-
		264		-	-	-
		15		-	-	-
	D	130	not realized	-	-	-
		240		-	-	-
		24		-	-	-
	E	96	not realized	-	+	+/-
		260		-	-	+/-
		238		-	+	+/-
		41		-	+	+/-
		123		-	+	+/-
09	A	22	-	+/-	-	+/-
		43	-	+/-	+	+/-
		72	-	+/-	-	+/-
		85	+	+/-	+	+/-
		116	-	+/-	+	+/-
	B	29	+	+/-	+	+/-
		70	+	+/-	-	+/-
		94	+	+/-	-	+/-
		126	-	+/-	-	+/-
		236	+	+/-	-	+/-
	C	20	-	-	-	-
		242	-	-	-	-
		247	-	-	-	-
	D	108	-	-	-	-
		180	-	-	-	-
		269	-	-	-	-
	E	67	-	-	+	+/-
		100	-	-	+	+/-
		114	-	-	+	+/-
		154	-	-	+	+/-
		248	-	-	+	+/-
10	A	40	-	+/-	+	+/-
		76	-	+/-	+	+/-
		90	-	+/-	-	+/-
		125	+	+/-	+	+/-
		220	-	+/-	+	+/-
	B	25	+	+/-	-	+/-
		38	+	+/-	-	+/-
		57	+	+/-	-	+/-
		81	+	+/-	-	+/-
		133	+	+/-	-	+/-
	C	87	-	-	-	-
		120	-	-	-	-
		179	-	-	-	-
	D	35	-	-	-	-
		175	-	-	-	-
		224	-	-	-	-
	E	88	+	-	+	+/-
		92	-	-	+	+/-
		196	-	-	+	+/-
		211	-	-	+	+/-
		214	**und**	-	+	+/-

			Xanthomonas (Xap)		Pseudomonas (Psp)	
Lab number	Codification of lot	Number of samples	Obtained results	Expected results	Obtained results	Expected results
12	A	69	+	+/-	-	+/-
		97	-	+/-	-	+/-
		121	-	+/-	+	+/-
		169	-	+/-	+	+/-
		251	-	+/-	+	+/-
	B	21	+	+/-	-	+/-
		77	+	+/-	**und**	+/-
		150	+	+/-	**und**	+/-
		218	+	+/-	-	+/-
		237	+	+/-	-	+/-
	C	34	-	-	-	-
		58	-	-	-	-
		157	-	-	-	-
	D	119	-	-	-	-
		124	-	-	-	-
		201	-	-	-	-
	E	93	**und**	-	+	+/-
		109	**und**	-	**und**	+/-
		132	**und**	-	+	+/-
		182	**und**	-	+	+/-
		254	-	-	+	+/-

INTER LABORATORY COMPARISON (ILC) REPORT

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