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

2018-ISTA-Rice-Ne-*Aphelenchoides besseyi*

Inter laboratory comparison (ILC) report*

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

Final

2018-ISTA-Rice-Ne-*Aphelenchoides besseyi*

Proficiency test: Detection of *Aphelenchoides besseyi* in Rice seeds

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** Original report signed and archived*

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Table des matières

Proficiency Test

Detection of *Aphelenchoides besseyi* in Rice seeds

1 PROFICIENCY TEST ORGANIZATION

The aim of this Proficiency Test was to verify the ability of laboratories to detect and identify *Aphelenchoides besseyi* in Rice seeds

The proficiency test includes 2 parts:

- Rice seeds samples to evaluate extraction and detection techniques described in the ISTA rules (ISTA Method 7-025).
- 6 tubes containing nematodes to implement identification techniques (name of genus and specie).

Schedule

Sending of samples	From 6 th of June to 28th of August 2018
Deadline to begin analysis	3 weeks after receipt
Deadline to send results	31 th of July 2018
Sending by GEVES of global report and individualized letters	31th of December 2018

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

On 10 participants registered for the proficiency test:

-1 of them was accredited for Method 7-025.

-9 were not accredited for this method.

1 laboratory did not receive samples after 2 attempts due to problems related to official documents requested and another one did not return results due to experimental problems.

Notation of results

The laboratories indicated:

- a quantitative and qualitative result for each sample and information about the method used.
- a name of nematodes for each tube for identification.

Composition of the sample panel

A panel of samples was sent to each laboratory, consisting of:

- samples of rice seeds contaminated or not with *Aphelenchoides besseyi* for the "detection" part
- tubes containing nematodes for the "identification" part

1) Detection on seed samples

20 samples of 250 rice seeds have been sent to each laboratory with different number of replicates depending on the level of contamination see table n°1.

Table n°1: Characteristics of samples

Level of contamination	Number of samples	Expected value
Healthy	4	Negative
Medium	12	Positive
High	4	Positive

Each sample was sent in a sealed bag.

2) Identification of nematodes

For the identification part, Professor Gerrit Karssen supplied us with 3 tubes containing different species of *Aphelenchoides* : *besseyi*, *fragaria* and *subtenuis*. These nematodes were provided to us dead and preserved in a conservation liquid.
We prepared 6 tubes containing 1 ml of solution with a minimum of 20 nematodes. The 6 tubes were sent coded by a letter (A to F).

The repartition of the panel is indicated in table n°2.

Table n°2: Composition of tube

Codification tube	Name of species
A	*Aphelenchoides besseyi*
B	*Aphelenchoides besseyi*
C	*Aphelenchoides besseyi*
D	*Aphelenchoides fragariae*
E	*Aphelenchoides subtenuis*
F	*Aphelenchoides subtenuis*

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

Four lots naturally contaminated with different levels (medium and high) sent from Indonesia and one healthy lot produced in France have been tested in four subsamples of 250 seeds by ISTA method the 17 th of August 2017. The results of the pretest are shown in table n°3.

Table n°3: Results of pretest

Lot number	**608**				**609**			
Subsample	**608/1**	**608/2**	**608/3**	**608/4**	**609/1**	**609/2**	**609/3**	**609/4**
Expected	**Low infected I**				**Low infected II**			
Number of A. *besseyi* by subsample	4	1	8	6	37	17	5	27
Average of nematods	4,75				21,5			
Total for 1000 seeds	**19**				**86**			

Lot umber	**610**				**611**			
Subsample	**610/1**	**610/2**	**610/3**	**610/4**	**611/1**	**611/2**	**611/3**	**611/4**
Expected	**High infected I**				**High infected II**			
Number of A. *besseyi* by sub sample	196	194	169	294	242	229	181	244
Average of nematods	213				224			
Total for 1000 seeds	**853**				**896**			

Lot number	**107**			
Subsample	**107/1**	**107/2**	**107/3**	**107/4**
Expected	**Healthy**			
Number of A. *besseyi* by sub sample	0	0	0	0
Average of nematods	0			
Total for 1000 seeds	**0**			

We chose:

-lot number 609 as the “medium” level with a nematode population lower than 100 per sub-sample.

- lot number 610 as the “high” level with a nematode population higher than 100 per sub-sample.

- lot 107 as healthy

We obtained therefore three different levels of infection.

Homogeneity Test

1) Detection on seed samples

Homogeneity test was done after packaging and just before sending. 10 extra samples of 250 seeds representing each contamination level were tested. The samples have been tested the 7 th of June 2018.

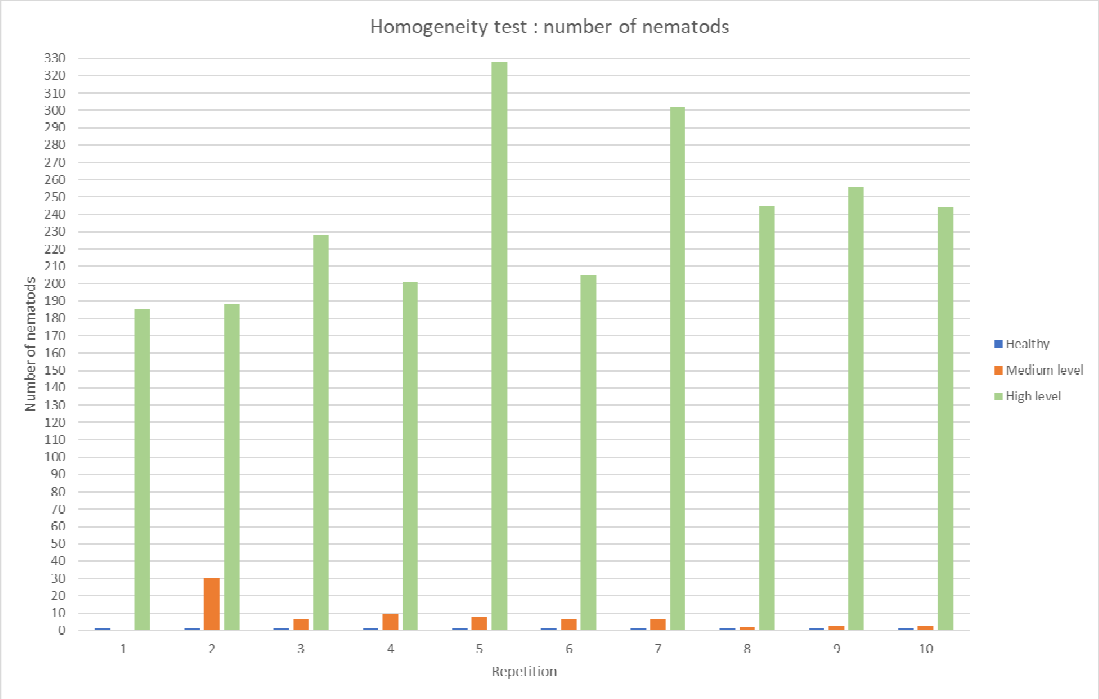
The raw data are given in Appendix. The table n°4 present the results and the graph n°1 the repartition of nematodes.

Table n°4: Results of homogeneity test.

Level of contamination	Expected result (detected/ not detected)	Average number of individuals detected: Quantitative result	mini-max of number of individuals detected	Qualitative result	Conformity
Healthy	not detected	0	0	$0^+/10$	In line
Medium	detected	9	2 -30	$9^+/9$*	In line
High	detected	238	185 -328	$10^+/10$	In line

* technical problem during the analysis

Graph n° 1: Distribution of nematodes



Conclusion of homogeneity test

- For healthy level: we obtained 0 positive samples. No false positive obtained.

- For medium level: we obtained 9 out of 9 positives samples, the number of nematodes varies between 2 to 30, the values are lower than 100.

- For the high level: we obtained 9 out of 9 positives samples, the number of nematodes varies between 185 to >300, the values are higher than 100.

The sample are homogeneous for qualitative results.

2) Identification of nematodes

Three repetitions for each tube were analyzed in single samples on 7 th of June because there is no intra-sample heterogeneity. Indeed, nematodes came from cultures and were individually selected by observation with a binocular microscope by experienced personnel during sample preparation, the results are given in table n°5.

Table n°5: Results of homogeneity test

Codification	Expected result:	Results obtained	**Conformity**
A	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*	**in line**
B	*A. besseyi*	*A. besseyi*	**in line**
C	*A. besseyi*	*A. besseyi*	**in line**
D	*A. fragariae*	*A. fragariae*	**in line**
E	*A. subtenuis*	*A. subtenuis*	**in line**
F	*A. subtenuis*	*A. subtenuis*	**in line**

Conclusion of homogeneity test

The results of homogeneity test are in line with the expected ones.

Stability Test

1) Detection on seed samples

Stability test has been started the 18th of July 2018. This test was carried out on 5 samples for each level of contamination. The last participant received the sample on 28 th of August 7 out of 9 all received before the date of stability test. Due to the expected biological stability of nematode infection we decided start the stability results before the last participants had received their samples.

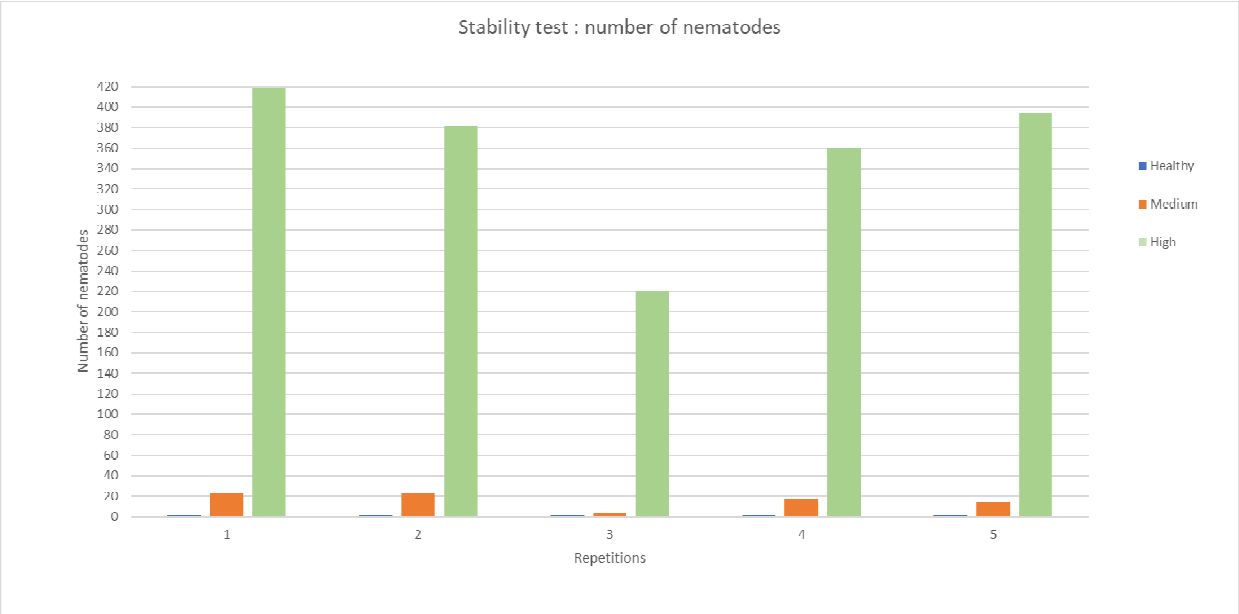
The raw data are given in appendix. The table n°6 present the results and the graph N°2 represent the distribution of nematodes.

Table n°6: Results of stability test.

Level of contamination	Expected result (detected/ not detected)	Average number of individuals detected: Quantitative result	mini-max of number of individuals detected	Qualitative result	Conformity
Healthy	not detected	0	0	$0^+/5$	in line
Medium	detected	16	3 -23	$5^+/5$	in line

High	detected	355	221 -419	$5^{+}/5$	in line

Graph n° 2: Distribution of nematodes



Conclusion of stability test

Stability of the lots has been confirmed. Test results are stable for the different levels of contamination, the results are all in accordance with the expected value

2) Identification of nematodes

This test was not carried out as there could be no change in the nematodes present in the tubes.

2 PROFICIENCY TEST RESULTS

Statistical analysis of data

Qualitative results for detection and identifications tests

- **Criteria of performance: diagnostic sensitivity –specificity for qualitative results**

The analysis was done by addition of the results of the 3 lots (healthy, medium and high level) according to the Standard NF EN ISO 16140 which expresses results as presence/absence. Results of medium and high level have been grouped for analysis.
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 agreements

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.

- **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. We use a qualitative rating system.

Quantitative results used for detection test only

⇨ BOXPLOT

Statistical analysis of results has been realized with the Boxplot tool. The "box plot" are graphical tools for visualizing key statistical measures. This tool compares the separate groups of similar numbers. The goal aims to give a good idea of center (use to median), of variability and to identify the aberrant values. Values given by participants have been compared to values obtained during homogeneity test for medium and high levels.

Analysis of data

Results for detection

⇨ Qualitative results

Raw data of all laboratories are given in appendix.

- **Specificity and sensibility**

Analysis of results of three levels 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°7.

Table n°7: Overview of qualitative results for each laboratory on the 3 levels

N° Lab	Healthy	Medium	High
01	$2^{+}/4$	$12^{+}/12$	$4^{+}/4$
03	$0^{+}/4$	$12^{+}/12$	$4^{+}/4$
04	$2^{+}/4$	$10^{+}/12$	$4^{+}/4$
06	$0^{+}/4$	$12^{+}/12$	$4^{+}/4$
07	$0^{+}/4$	$4^{+}/12$	$4^{+}/4$
08	$0^{+}/4$	$12^{+}/12$	$4^{+}/4$
09	$1^{+}/4$	$11^{+}/11$**	$4^{+}/4$
10	$1^{+}/4$	$12^{+}/12$	$4^{+}/4$

** : received 15 out of 16 samples sent

All laboratories identified the 4 high infected samples. False negative results were only observed for medium level. False positive results were observed for the healthy level.

Criteria of performance as specificity per lab are indicated in Table n°8. Medium and high-levels results have been grouped for analysis.

Table n°8: Criteria of performance for each laboratory

Lab number	Sensitivity	Specificity	Accuracy
01	100%	50%	90%
03	100%	100%	100%
04	63%	50%	60%
06	100%	100%	100%
07	25%	100%	40%
08	100%	100%	100%
09	100%	75%	95%
10	100%	75%	95%

Evaluation of performance criteria of participants:

Three laboratories obtained 100% of sensitivity (no false negative), 100% of specificity (no false positive).

Four laboratories obtained false negative and/or false positive results.

Conclusion:

The healthy lot was produced in France, *A besseyi* is not present on French territory. It was sampled and prepared before the contaminated lots and on geographically different sites. It can't have had any cross-contamination. Positive results for healthy lots are therefore considered as false positive.

- **Z-score-computations and rating system**

Rules of decision:

A corresponds to no false positive in healthy level and the number of positive samples obtained is equal to the number of positive expected.

B using for 0 false positive in healthy level and one sample less than expected is accepted for medium level.

C using for 0 false positive in healthy level and two samples less than expected is accepted for medium level.

BMP (Below Minimum Performance) corresponds to a not expected result with a false positive in healthy level or more than 3 samples of deviation from the expected for the medium level.

The results are presented in table n° 9, it contains 2 parts:

A rating for all laboratories except laboratory 9

A rating for laboratory 9 due to a different number of samples received

Distribution of rating is presented figure n°1

Table n°9: Computations of laboratories and rating.

Rating for all laboratories (except Lab 9)

Rating for qualitative SH PTs — Change any value in a yellow cell

Minimum requirements for A rating :

	Healthy lot		High and medium levels lot
Max # of pos reps:	0	and Min # of pos reps:	16

Minimum requirements for B rating :

	Healthy lot		High and medium levels lot
Max # of pos reps:	0	and Min # of pos reps:	15

Minimum requirements for C rating :

	Healthy lot		High and medium levels lot
Max # of pos reps:	0	and Min # of pos reps:	14

Rating	Lab	Healthy lot # of pos reps	High and medium levels lot # of pos reps
BMP	1	2	16
A	3	0	16
BMP	4	2	14
A	6	0	16
BMP	7	0	8
A	8	0	16
BMP	10	1	16

Rating for laboratory °9

Rating for qualitative SH PTs — Change any value in a yellow cell

Minimum requirements for A rating :

	Healthy lot		High and medium levels lot
Max # of pos reps:	0	and Min # of pos reps:	15

Minimum requirements for B rating :

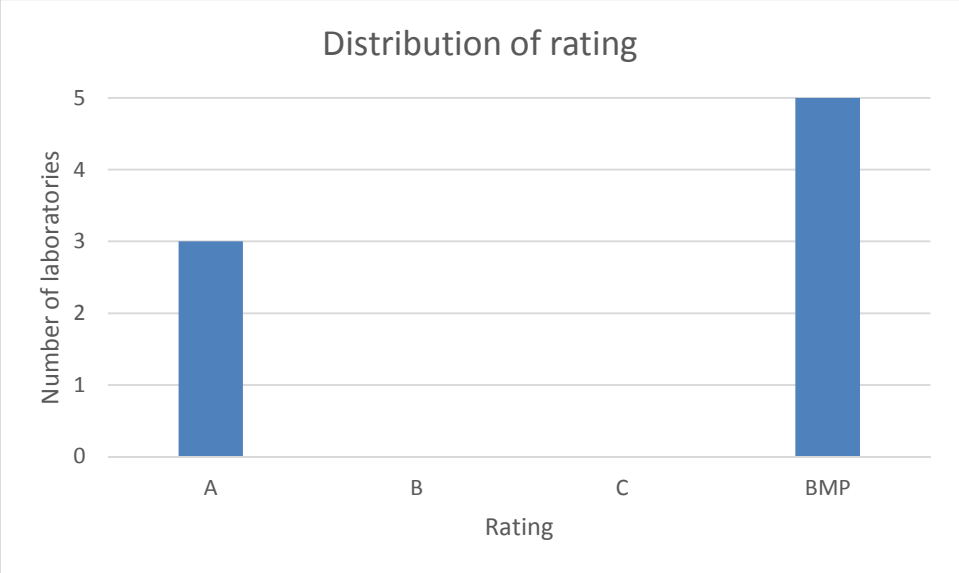
	Healthy lot		High and medium levels lot
Max # of pos reps:	0	and Min # of pos reps:	14

Minimum requirements for C rating :

	Healthy lot		High and medium levels lot
Max # of pos reps:	0	and Min # of pos reps:	13

Rating	Lab	Healthy lot # of pos reps	High and medium levels lot # of pos reps
BMP	9	1	15

Figure n°1: Distribution of rating



The distribution of rating is divided between the letter A and BMP.
Three laboratories achieved an A rating and five achieved a BMP rating.
The BMP rating is due:

- To a false positive in the healthy lot for 4 laboratories
- or/and a lower number of positive samples

⇨ **Quantitative results**

The quantitative analysis was carried out using the box plot statistical tool, this study is informative and will not be subject to evaluation.

- **Medium level**

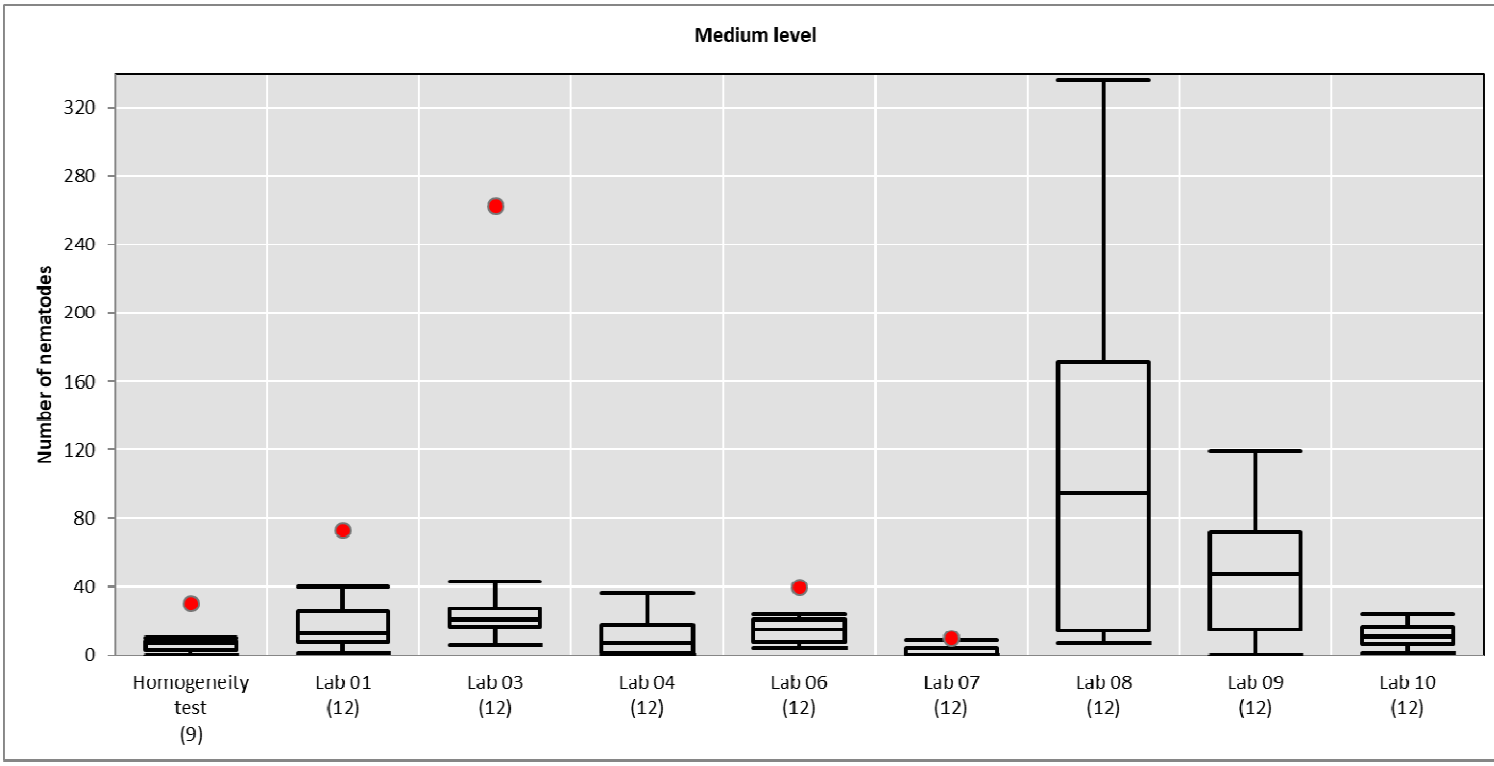
All results are given in Table n°10 for each laboratory

Table n°10: Number of nematodes found

Sample number	Lab number								
	01	03	04	06	07	08	09	10	Homogeneity test
1	11	6	6	8	0	59	0	10	-
2	1	27	19	14	10	136	85	7	30
3	8	43	8	7	9	7	72	13	7
4	24	16	36	15	0	51	23	10	10
5	14	25	0	4	0	8	17	3	8
6	73	10	36	16	6	336	9	18	7
7	6	263	3	22	0	130	56	16	7
8	8	20	12	24	0	314	39	5	2
9	31	17	0	15	3	213	9	11	3
10	40	22	1	40	0	158	119	21	3
11	21	18	17	4	0	8	73	24	
12	7	28	1	20	0	16	59	1	
Average	20	41	12	16	2	120	47	12	9
Mini -maxi	1-73	6 - 263	1 - 36	4 - 40	3 -10	7 - 314	9 - 119	1 -24	2 -30

The figure n°2 present the dispersion of the 129 values obtained, using box plot.

Figure n°2: BoxPlot



The dispersion is similar to the one of the homogeneity test for 6 laboratories and 2 laboratories obtained a more extensive dispersion (Lab 08 and 09). It means that the score of nematodes detected is higher than expected, they have a tendency to overestimate the number of nematodes.

- **High level**

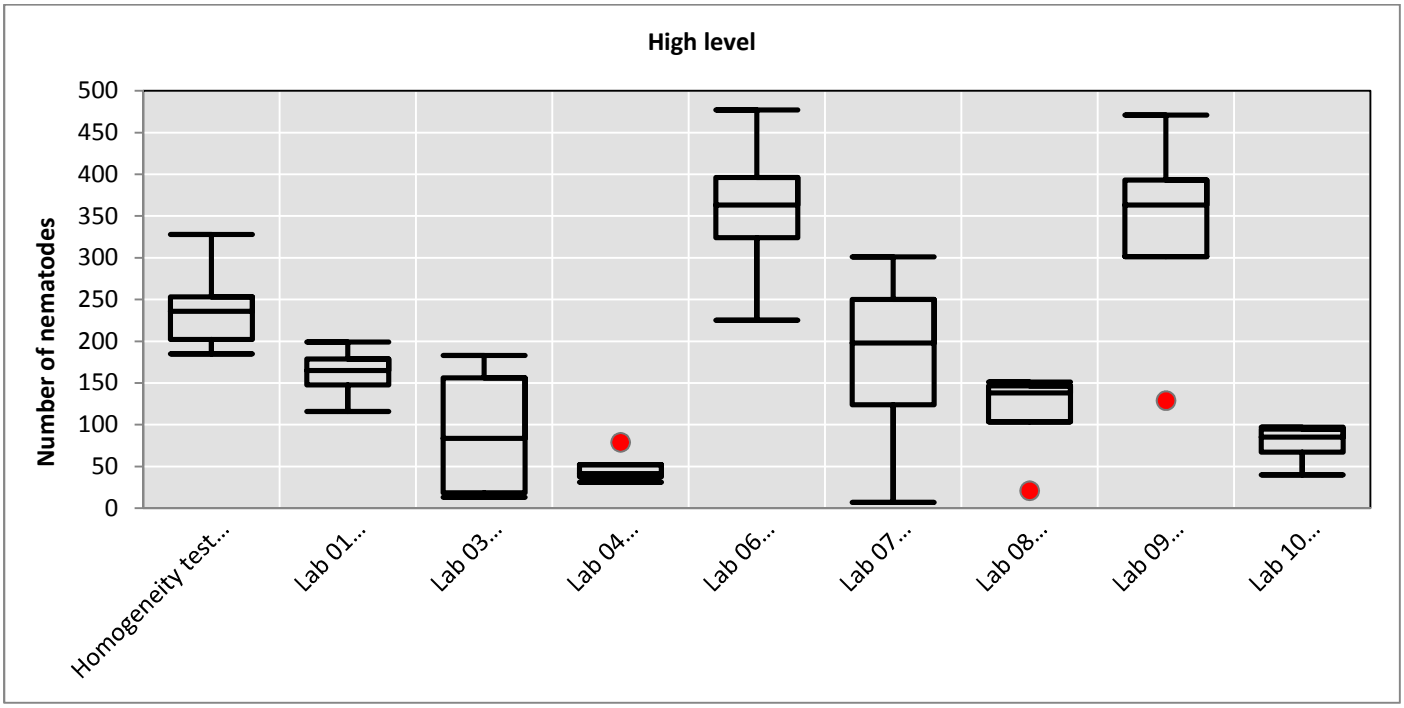
All results are given in Table n°5 for each participant

Table n°5: Number of nematodes found per participant

Sample number	Lab number								
	01	03	04	06	07	08	09	10	Homogeneity test
1	199	183	79	225	301	151	129	97	185
2	116	20	43	357	233	145	359	76	188
3	172	147	31	369	163	21	471	40	228
4	158	13	40	477	7	131	367	94	201
Average	161	91	48	357	176	112	332	77	201
Mini -maxi	116-199	13 - 183	31 - 79	225 - 477	7 -301	21 - 151	129 - 471	40 -97	185 - 228

The figure n°3 present the dispersion of the 36 values obtained by using box plot, without changing the scale.

Figure n°3: BoxPlot



On the graph, we can observe that 3 out of 32 values are outliers (10%), one value for the laboratory (Lab 04, 08 and lab 09). There is more variability in the results of the high than medium lot.

Six laboratories have results with a lower number of nematodes than the homogeneity test. Lab 4, 8 and 10 have a particular tendency to underestimate the number of nematodes.

Results for identification

- **Diagnostic specificity and sensitivity**

Raw data of all laboratories are given in Appendix. One laboratory didn't return the results.

The performance criteria were assessed separately as 2 entities: *A. besseyi* (3 tubes) and other than *A. besseyi* regrouping 2 tubes of A. *subtenuis* +1 tube of A. *fragariae*.

		Aphelenchoides besseyi			Other *Aphelenchoides*		
Tube codification	Name of nematodes	**Sensitivity**	**Specificity**	**Accuracy**	**Sensitivity**	**Specificity**	**Accuracy**
A	*A . besseyi*	X		X		X	X
B	*A . besseyi*	X		X		X	X
C	*A . besseyi*	X		X		X	X
D	*A . fragariae*		X	X	X		X
E	*A . subtenuis*		X	X	X		X
F	*A . subtenuis*		X	X	X		X

Decision rule for statistical tools:
We accept as correct the return information of laboratory for "other than *A. besseyi*".

Aphelenchoides genus, *Aphelenchoides* sp. or the correct species name.

The use of "sp." has been interpreted as "another *Aphelenchoides* whose species is unknown but not identified as *Aphelenchoides besseyi*".

Evaluation of performance

The criteria of performance as specificity per lab are indicated in Table n°6.

Table n°6: Criteria of performance for each laboratory

Lab number	*A . besseyi*			Other than *A . besseyi*		
	Sensitivity	**Specificity**	**Accuracy**	**Sensitivity**	**Specificity**	**Accuracy**
01	0%	100%	50%	100%	0%	50%
03	100%	67%	83%	67%	100%	83%
05	100%	67%	83%	67%	100%	83%
06	100%	100%	100%	100%	100%	100%
07	100%	100%	100%	100%	100%	100%
08	67%	100%	83%	100%	67%	83%
09	33%	100%	67%	100%	33%	67%
10	33%	67%	50%	67%	33%	50%

Two laboratories (Lab 06 and 07) obtained 100% for identification of *Aphelenchoides* species.

Identification at 83%: 3 laboratories obtained this rate:
Two laboratories Lab 03 and 05 identified the coded tube D as *A. besseyi*.
The laboratory (Lab 08) identified the tube coded A as not *A. besseyi*.

Identification at 67%: 1 laboratory obtained this rate

The laboratory (Lab 09) identified 2 tubes coded B and C as not *A*. *besseyi*. It makes false negative on identification of *A*. *besseyi* and it indicates 4 different species names.

Identification at 50%: 2 laboratories obtained this rate
The laboratory (Lab 01) identified 3 tubes coded A; B and C as not *A*. *besseyi*. It makes false negative on identification of *A*. *besseyi*.

The laboratory (Lab 10) identified 2 tubes coded A; B as not *A*. *besseyi*. It makes false negative on identification of *A*. *besseyi* and identified 1 tube coded D as *A*. *besseyi*, it makes a false positive on identification of *A*. *besseyi.*

Conclusion:

Concerning the identification of tubes, 2 laboratories correctly identified all tubes.
The lab 01 didn't identify 0 out of 3 tube of *Aphelenchoides besseyi*. It indicates "sp." it means that it is an *Aphelenchoides* species, the ISTA method requires a more precise identification because it is a plant pathogenic nematode for the rice.
.
The 3 false-positive results obtained by the laboratories (Lab 03; 05;10) relate to the identification of the D tube.
There has been confusion of identification between A. *fragariae* and *besseyi*, these 2 nematodes have some morphologically similar criteria.

There was no problem to see that the *A*. *subtenuis* is morphologically different that *A*. *besseyi*.

- **Z-score-computations and rating system**

Rules of decision:
We adapted the tools for identification part.
The column about healthy lot was not used in this case.
We used 2 columns: one corresponds to identification of *A*. *besseyi* and the other regrouping tube other than *A*. *besseyi*.
A corresponds to an expected result: 3 tubes of *A*. *besseyi* or 3 tubes other than *A*. *besseyi* depending of the column.
B corresponds to an expected result: 2 tubes of *A*. *besseyi* or 2 tubes other than *A*. *besseyi.*
C corresponds to an expected result: 1 tube of *A*. *besseyi* or 1 tube other than *A. besseyi*.
BMP (Below Minimum Performance) corresponds to a not expected result for all tubes.

The final rating represents the minimum obtained rating.

Rating for qualitative SH PTs — Change any value in a yellow cel

Minimum requirements for A rating :

	Healthy lot		A. besseyi		Other than A. besseyi
Max # of pos reps:		and n # of pos rep	3		3

Minimum requirements for B rating :

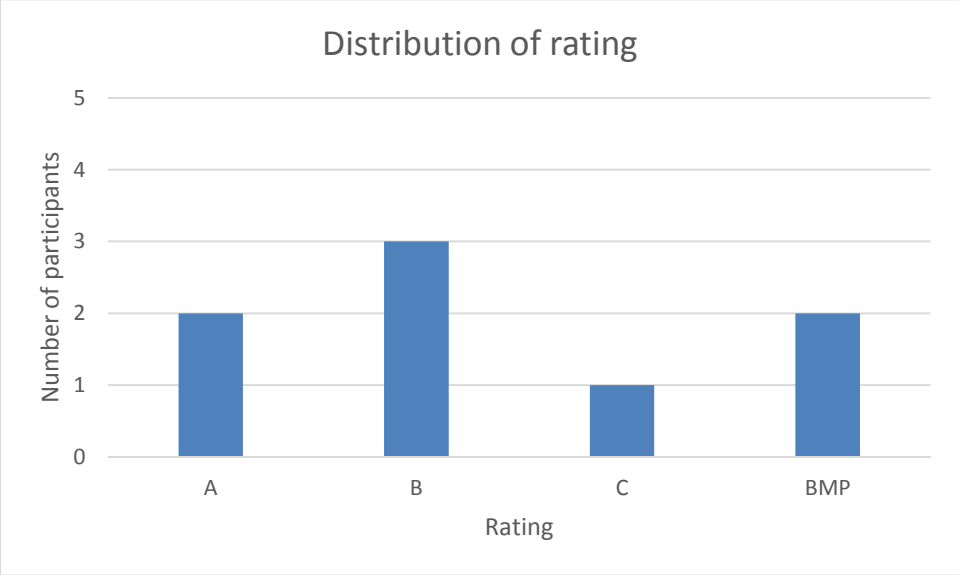
	Healthy lot		A. besseyi		Other than A. besseyi
Max # of pos reps:		and n # of pos rep	2		2

Minimum requirements for C rating :

	Healthy lot		A. besseyi		Other than A. besseyi
Max # of pos reps:		and n # of pos rep	1		1

		Healthy lot		A. besseyi		other than A. besseyi
Ratin	Lab	# of pos reps		# of pos reps		# of pos reps
BMP	1			0		3
B	3			3		2
B	5			3		2
A	6			3		3
A	7			3		3
B	8			2		3
BMP	9			1		0
C	10			1		2

Figure n°7: Distribution of rating



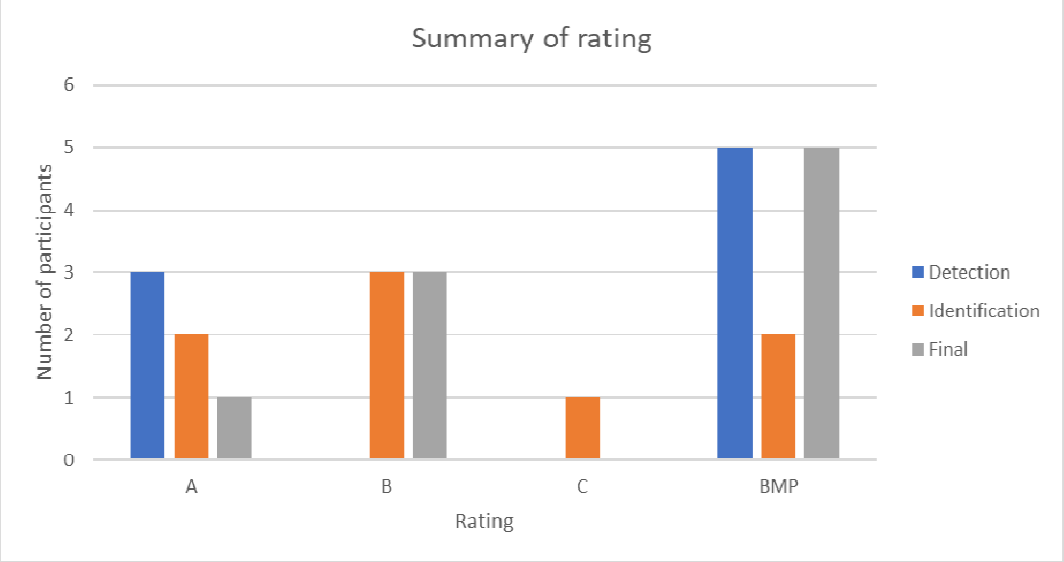
At the final, two laboratories achieved an A rating, three laboratories achieved a B rating and one achieved C and the last achieved an BMP. The BMP rating is due a lack of identification of tubes containing the *A besseyi*.

3 CONCLUSION

The table is a summary of the different results

Lab number	Detection			Identification			Final rating
	% Accuracy	Deviation	Rating	% Accuracy	Deviation	Rating	
01	91	2 out of 4 false positive samples	BMP	50	3 out of 6 false positive	BMP	BMP
03	100		A	83	1 out of 6 false positive	B	B
04	56	2 out of 4 false positive samples + 2 out of 16 false negative samples	BMP				BMP
05				83	1 out of 6 false positive	B	B
06	100		A	100		A	A
07	71	8 out of 16 false negative samples	BMP	100		A	BMP
08	100		A	83	1 out of 6 false negative	B	B
09	95	1 out of 4 false positive samples	BMP	67	2 out of 6 false negative	BMP	BMP
10	95	1 out of 4 false positive samples	BMP	50	1 out of 6 false positive + 2 out of 6 false negative	C	BMP

The scores obtained on the detection part are more penalizing than for the identification.
One laboratory obtained an A rating and 3 obtained an B rating and 5 obtained the BMP rating is due to false positive samples and/or 50% of false negative samples.



It is the first PT organized to detect *A. besseyi*, only one lab obtained an A rating, showing that criteria of identification for *A. besseyi* are very important to check during testing.

Acknowledgment
This PT was made possible due to a fruitful international collaboration. The organizer thanks the laboratory of the ANSES based in Rheu for its commitment during all the proficiency test, Professor Gerrit Karssen for giving us the opportunity to make an identification game and Ms. Fadhilah Siti Hila for the supply of contaminated seeds without which the PT would not have been organized.

Appendix :
1) Raw data for detection part

N° Lab	Level of contamination	Sample number (participant)	Qualitative results			Quantitative results
			Obtained results	Expected results	NB positive/total	
01	Healthy	14	-	-	**2⁺/4**	0
		50	-	-		0
		155	**+**	-		4
		195	**+**	-		7
	Medium	27	+	+	12⁺/12	11
		31	+	+		1
		36	+	+		8
		69	+	+		24
		107	+	+		14
		123	+	+		73
		166	+	+		6
		204	+	+		8
		216	+	+		31
		217	+	+		40
		247	+	+		21
		256	+	+		7
	High	72	+	+	4⁺/4	199
		183	+	+		116
		227	+	+		172
		259	+	+		158
03	Healthy	20	-	-	0⁺/4	0
		65	-	-		0
		133	-	-		0
		260	-	-		0
	Medium	3	+	+	12⁺/12	6
		10	+	+		27
		32	+	+		43
		67	+	+		16
		76	+	+		25
		108	+	+		10
		119	+	+		263
		144	+	+		20
		149	+	+		17
		194	+	+		22
		219	+	+		18
		257	+	+		28
	High	23	+	+	4+/4	183
		57	+	+		20
		93	+	+		147
		161	+	+		13
04	Healthy	88	-	-	**2⁺/4**	0
		106	**+**	-		3
		152	-	-		0
		269	**+**	-		2
	Medium	30	+	+	**10⁺/12**	6
		33	+	+		19
		100	+	+		8
		101	+	+		36
		136	-	+		0
		146	+	+		36
		157	+	+		3
		159	+	+		12
		169	-	+		0
		246	+	+		1
		258	+	+		17
		264	+	+		1
	High	24	+	+	4⁺/4	79
		95	+	+		43
		126	+	+		31
		233	+	+		40

N° Lab	Level of contamination	Sample number (participant)	Qualitative results			Quantitative results
			Obtained results	Expected results	NB positive/total	
06	Healthy	29	-	-	0+/4	0
		49	-	-		0
		61	-	-		0
		158	-	-		0
	Medium	4	+	+	12+/12	8
		22	+	+		14
		130	+	+		7
		138	+	+		15
		170	+	+		4
		177	+	+		16
		187	+	+		22
		203	+	+		24
		209	+	+		15
		224	+	+		40
		226	+	+		4
		272	+	+		20
	High	51	+	+	4+/4	225
		105	+	+		357
		116	+	+		369
		174	+	+		477
07	Healthy	59	-	-	0^{+}/4	0
		83	-	-		0
		164	-	-		0
		244	-	-		0
	Medium	25	-	+	**4^{+}/12**	0
		66	+	+		10
		77	+	+		9
		84	-	+		0
		103	-	+		0
		115	+	+		6
		129	-	+		0
		228	-	+		0
		254	+	+		3
		262	-	+		0
		263	-	+		0
		278	-	+		0
	High	55	+	+	4^{+}/4	301
		79	+	+		233
		86	+	+		163
		206	+	+		7
08	Healthy	151	-	-	0+/4	0
		156	-	-		0
		250	-	-		0
		251	-	-		0
	Medium	13	+	+	12+/12	59
		18	+	+		136
		35	+	+		7
		78	+	+		51
		117	+	+		8
		150	+	+		336
		190	+	+		130
		196	+	+		314
		199	+	+		213
		207	+	+		158
		261	+	+		8
		279	+	+		16
	High	2	+	+	4+/4	151
		6	+	+		145
		60	+	+		21
		165	+	+		131

N° Lab	Level of contamination	Sample number (participant)	Qualitative results			Quantitative results
			Obtained results	Expected results	NB positive/total	
09	Healthy	17	-	-	**1+/4**	0
		75	-	-		0
		234	-	-		0
		243	**+**	-		211
	Medium	21			11+/11	
		56	+	+		85
		87	+	+		72
		111	+	+		23
		121	+	+		17
		125	+	+		9
		163	+	+		56
		188	+	+		39
		202	+	+		9
		229	+	+		119
		232	+	+		73
		277	+	+		59
	High	19	+	+	4+/4	129
		182	+	+		359
		215	+	+		471
		267	+	+		367
10	Healthy	82	-	-	1+/4	0
		141	-	-		0
		143	-	-		0
		214	+	-		7
	Medium	9	+	+	12+/12	10
		58	+	+		7
		98	+	+		13
		114	+	+		10
		21	+	+		3
		127	+	+		18
		153	+	+		16
		162	+	+		5
		179	+	+		11
		193	+	+		21
		223	+	+		24
		253	+	+		1
	High	47	+	+	4+/4	97
		99	+	+		76
		197	+	+		40
		241	+	+		94

N° Lab	Level of contamination	Sample number (participant)	Qualitative results			Quantitative results
			Obtained results	Expected results	NB positive/total	
Homogeneity test	Healthy	285	-	-	0^+/10	0
		286	-	-		0
		287	-	-		0
		288	-	-		0
		290	-	-		0
		294	-	-		0
		297	-	-		0
		316	-	-		0
		322	-	-		0
		323	-	-		0
	Medium	298	+		9^+/10	Technical problem
		299	+	+		30
		302	+	+		7
		303	+	+		10
		307	+	+		8
		311	+	+		7
		312	+	+		7
		315	+	+		2
		319	+	+		3
		324	+	+		3
	High	280	+	+	10^+/10	302
		283	+	+		245
		292	+	+		256
		295	+	+		244
		300	+	+		205
		304	+	+		328
		308	+	+		228
		310	+	+		201
		317	+	+		185
		318	+	+		188
Stability test	Healthy	293	-	-	0^+/5	0
		301	-	-		0
		305	-	-		0
		309	-	-		0
		314	-	-		0
	Medium	281	+	+	5^+/5	23
		289	+	+		22
		306	+	+		3
		313	+	+		17
		320	+	+		14
	High	282	+	+	5^+/5	419
		284	+	+		382
		291	+	+		221
		296	+	+		360
		321	+	+		394

2) Raw data for identification part

Lab number	Tube codification	Expected results	Obtained results
01	A	*Aphelenchoides besseyi*	*Aphelenchoides* sp. (13)
	B	*Aphelenchoides besseyi*	*Aphelenchoides* sp. (4)
	C	*Aphelenchoides besseyi*	*Aphelenchoides* sp. (13)
	D	*Aphelenchoides fragariae*	0
	F	*Aphelenchoides subtenuis*	0
	F	*Aphelenchoides subtenuis*	*Aphelenchoides* sp. (3)
03	A	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	B	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	C	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	D	*Aphelenchoides fragariae*	*Aphelenchoides besseyi*
	F	*Aphelenchoides subtenuis*	*Aphelenchoides subtenuis*
	F	*Aphelenchoides subtenuis*	*Aphelenchoides subtenuis*
05	A	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	B	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	C	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	D	*Aphelenchoides fragariae*	*Aphelenchoides besseyi*
	F	*Aphelenchoides subtenuis*	negative
	F	*Aphelenchoides subtenuis*	negative
06	A	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	B	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	C	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	D	*Aphelenchoides fragariae*	*Aphelenchoides fragariae*
	F	*Aphelenchoides subtenuis*	*Aphelenchoides subtenuis*
	F	*Aphelenchoides subtenuis*	*Aphelenchoides subtenuis*
07	A	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	B	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	C	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	D	*Aphelenchoides fragariae*	*Aphelenchoides* genus
	F	*Aphelenchoides subtenuis*	*Aphelenchoides* genus
	F	*Aphelenchoides subtenuis*	*Aphelenchoides* genus
08	A	*Aphelenchoides besseyi*	*A. ritzemabosi*
	B	*Aphelenchoides besseyi*	*A. besseyi*
	C	*Aphelenchoides besseyi*	*A. besseyi*
	D	*Aphelenchoides fragariae*	*Aphelenchoide* s sp.
	F	*Aphelenchoides subtenuis*	*A. subtenuis*
	F	*Aphelenchoides subtenuis*	*A. subtenuis*
09	A	*Aphelenchoides besseyi*	*Aphelenchoides besseyi* Christie
	B	*Aphelenchoides besseyi*	*Aphelenchoides blastophthorus* Franklin
	C	*Aphelenchoides besseyi*	*Aphelenchoides sphaerocephalus* Goodey
	D	*Aphelenchoides fragariae*	*Aphelenchoides longiurus* Das
	F	*Aphelenchoides subtenuis*	Negative
	F	*Aphelenchoides subtenuis*	*Aphelenchoides martinii* Ruehm
10	A	*Aphelenchoides besseyi*	*Aphelenchoides ritzemabosi*
	B	*Aphelenchoides besseyi*	*Aphelenchoides ritzemabosi*
	C	*Aphelenchoides besseyi*	*Aphelenchoides besseyi*
	D	*Aphelenchoides fragariae*	*Aphelenchoides besseyi*
	F	*Aphelenchoides subtenuis*	*Aphelenchoides subtenuis*
	F	*Aphelenchoides subtenuis*	*Aphelenchoides subtenuis*

INTER LABORATORY COMPARISON (ILC) REPORT

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Variety and Seed Study and control Group

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