ISTA Secretariat
Richtiarkade 18, 8304 Wallisellen, Switzerland
Phone: +41 44 838 60 00 | Fax: +41 44 838 60 01
Email: ista.office@ista.ch
www.seedtest.org

Inter laboratory comparison (ILC) report*

ISTA PT21-SH 7-031

ISTA Proficiency test: Detection of *Ditylenchus dipsaci* in Alfalfa seeds

*Original report signed and archived

COORDINATION	FULL NAME	POSITION
Coordinator of ILC	Isabelle Sérandat	Seed health manager GEVES-SNES, FR
Organization	Ms Corinne Sahuguede	Proficiency test organization GEVES-SNES, FR
Samples preparation	Ms Hélena Beduneau	Nematology analyst GEVES-SNES, FR
Validation of report's diffusion	Ms Valérie Grimault	Head phytopathology laboratory GEVES-SNES, FR

Table of Content

PROFICIENCY TEST ORGANIZATION

The aim of this Proficiency Test was to evaluate the ability of laboratories to detect and identify *Ditylenchus dipsaci* in Alfalfa seeds.

The proficiency test includes 2 parts:

- Part1: Alfalfa seed samples to assess the ability of the participant to correctly extract samples and detect *D. dipsaci*.
- Part 2: 6 tubes containing nematodes to evaluate identification capacity of the participating laboratories.

Schedule

Sending of samples	4 th of October 2021
Deadline to begin analysis	2 weeks after receipt
Deadline to send results	15 th of November 2021 Report to 15 th of December
Sending by GEVES of global report and individualized letters	28th of February 2022 **Delayed due to late delivery of the results**

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

All laboratories are voluntary. 8 out 9 laboratories send the results, one laboratory doesn't send these results.

We choice to include the results of the organizer for information.

Notation of results

The laboratories indicated:

- Part 1: a qualitative result for each sample and information about the method used.
- Part 2: the result is for each tube as *Ditylenchus dipsaci* identified or other nematodes detected.

Composition of the sample panel

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

- 12 samples of Alfalfa seeds contaminated or not with *Ditylenchus dipsaci* for the "Part 1".
- 6 tubes containing nematodes for the "Part 2" part.

1) Part 1: extraction on seed samples

12 samples of 100 grams of seeds have been sent to each laboratory with different number of replicates depending on the level of contamination as shown in table n°1.

Table n°1: Characteristics of samples

Codification	Level of contamination	Number of samples	Expected value
H	Healthy	2	Negative
MI	Medium infected	5	Positive
MC	Medium contaminated	3	Positive
HC	Highly contaminated	2	Positive

Each sample was sent in a red pot.

2) Part 2: identification of nematodes

For the identification exercise: 3 species of nematodes have been chosen:

Ditylenchus dipsaci and *Heterodera schachtii* supplied by the varietal resistance team of the GEVES pathology laboratory.

Ditylenchus destructor, from nematode breeding at GEVES.

We prepared 6 tubes containing 1 ml of water with a minimum of 100 nematodes. The 6 tubes were coded by a letter (A to F).

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

Table n°2: Nematode species contained in codified tubes

Codification	Name of species
A	*Ditylenchus destructor*
B	*Heterodera schachtii*
C	*Ditylenchus dipsaci*
D	*Heterodera schachtii*
E	*Ditylenchus destructor*
F	*Ditylenchus dipsaci*

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. These results were confirmed by the stability test.

Pretest

The medium infected lot (MI) contains seeds infected with *Ditylenchus dipsaci* and presents different larval stages (juveniles and adults), while the healthy (H) lot contains healthy seeds. MI and H lots have been tested for this proficiency test.

The infected lot (MI) has been tested in 10 th of June 2021 on 10 samples of 100g of Alfalfa seeds, we extracted between 2 and 120 alive *Ditylenchus dipsac*i nematodes and other nematodes identified as *Aphelenchoides* spp. Below the results of extraction of this lot in table n°3.

Table n°3: Result of pre-test

Sample Number	Number of *Ditylenchus dipsaci*	Number of *Aphelenchoides* spp.
1	2	2
2	36	7
3	91	11
4	120	41
5	3	3
6	8	1
7	98	1
8	19	1
9	20	1
10	52	1

We created:

1. Healthy samples from the healthy seed lot.
2. A medium level with 2 types of contaminations:

   MI = naturally infected seed lot

   MC= artificially contaminated by fishing and adding 10 adult nematodes from breeding to the healthy seed samples in a red pot to obtain a lot with a low contamination.

3. A high level of contamination:

   HC = we added a minimum of 100 nematodes from breeding in 2 points of deposits in the form of drops in a healthy seed sample.

We obtained therefore four different levels (H, MI, MC and HC).

Homogeneity Test

1) Part 1: extraction on seed samples

Homogeneity test was done after packaging and just before sending of the samples. 10 extra samples of 100 grams of alfalfa seeds representing each contamination level were tested. The samples have been tested the 10 th of August 2021.

The table n°4 present the results of homogeneity test.

Table n°4: Results of homogeneity test.

Codification	Level of contamination	Expected result (detected/ not detected)	Qualitative result	Conformity
H	Healthy	not detected	$0^{+}/10$	Conform
MC	Medium contaminated	detected	$7^{+}/10$	Conform
MI	Medium infected	detected	$10^{+}/10$	Conform
HC	Highly contaminated	detected	$10^{+}/10$	Conform

Conclusion of homogeneity test

- For healthy (H): we obtained 0 positive samples. No false positive obtained.

- For the medium levels:
  MC: we obtained 7 out of 10 positives samples.
  MI: we obtained 10 out of 10 positives samples.

- For highly contaminated (HC): we obtained 10 out of 10 positives samples. No false negative was obtained.

The H, MI and HC samples are homogeneous for qualitative results. We note a variability for the MC lot (Medium contaminated), not all samples are detected. This level was close to the LOD (limit of detection) of the method.

2) Part 2: identification of nematodes

We didn't do homogeneity tests on identification tubes. The nematodes were multiplied independently, species by species., and added in each tube. From a population of each species we determined the concentration of the population and diluted it to obtain about 50 nematodes in 1 ml.
After this dilution, 10 drops of 100 ul for each genus-species were taken to ensure the correct concentration.

Stability Test

1) Part 1: extraction on seed samples

According to the homogeneity test, we extrapolate the expected results of the stability test.

Stability test has been started the 16th of November 2021. This test was carried out on 3 samples for each level of contamination excepted for the medium infected where 10 samples were analyzed.

For the" Medium contaminated" (MC), the result of homogeneity test was used for the computation of probability to obtain contaminated samples out of tested samples. The percentage of contamination obtained with homogeneity test was 0.0024% (computed % in sample) corresponding to 7 positive samples out of 10 (Fig.1). Therefore, the probability at 5% to obtain positive samples was from 1 to 3 (Fig.2) in the stability test.

Figure 1: Results of medium level with the Seedcalc8 software.

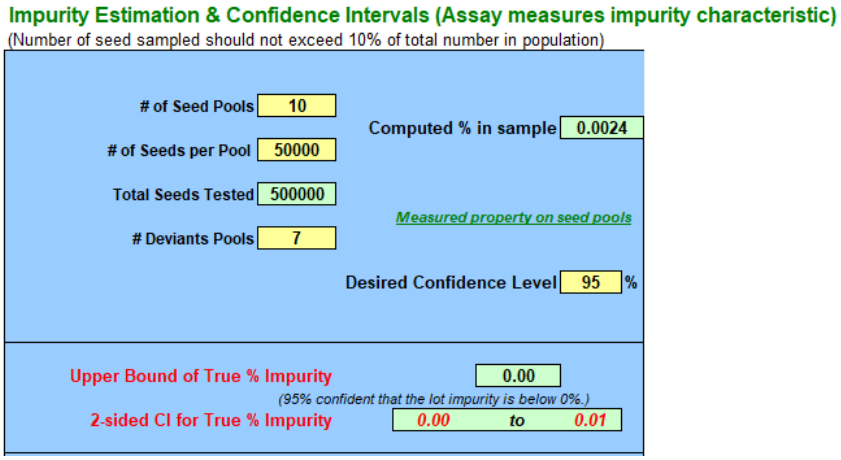
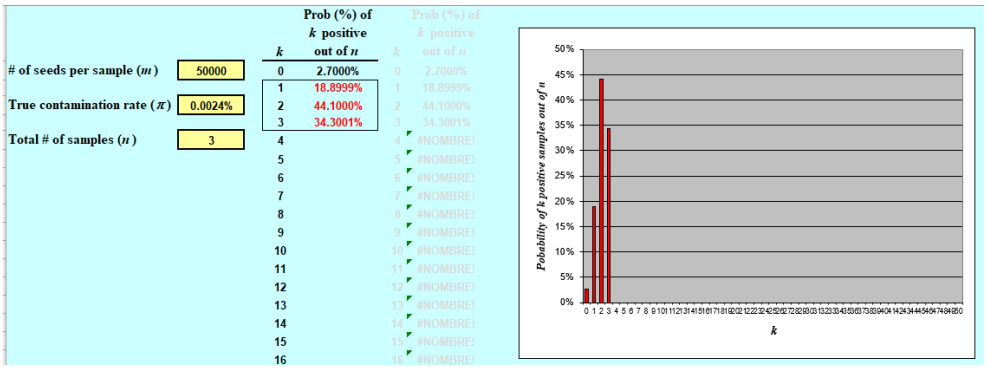


Figure 2: Expected number of infected samples according to infection rate.



The table n°5 present the results and the conformity of the stability test.

Table n°5: Results of stability test.

Codification	Level of contamination	Expected result		Obtained result	Conformity
		detected/ not detected	Qualitative result		
H	Healthy	not detected	$0^{+}/3$	$0^{+}/3$	Conform
MC	Medium contaminated	detected	1 to $3^{+}/3$	$3^{+}/3$	Conform
MI	Medium infected	detected	$10^{+}/10$	$10^{+}/10$	Conform
HC	Highly contaminated	detected	$3^{+}/3$	$3^{+}/3$	Conform

Conclusion of stability test

- For healthy level, we obtained 0 positive samples. No false positive obtained.
- For medium levels, we obtained:
  MC: $3^{+}/3$ is in accordance with the expected results (1 to 3).
  MI: $10^{+}/10$ is in accordance with the expected results
- For highly contaminated (HC): we obtained: HC: $3^{+}/3$

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) Part 2: identification of nematodes

A visual check was carried out on 13th of December to check that the morphological criteria were intact. The lethality of the nematodes was observed, nevertheless the morphological criteria (dimensions, stylet, tail shape, etc.) allowing identification were present.

PROFICIENCY TEST RESULTS

Statistical tools

⇨ **Diagnostic sensitivity and 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 + (infected 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 infected 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 infected samples over the number tested from the % determined with Seedcalc8 tool.

⇨ **Rating system**

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.

- **Rules of decision for extraction part**

A corresponds to no false positive in healthy level and no false negative in high levels ($2^+/2$ for the HC and $5^+/5$ for the MI) and the number of positive samples obtained is equal to the number of positive expected for the Medium contaminated (1 to $3^+/3$) corresponding to an expected using probability of 5%.

B using for 0 false positive in healthy level and one sample less than expected is accepted for high levels ($2^+/2$ for the high and $4^+/5$ for the Medium Infected) and the number of positive samples obtained

is equal to the number of positive expected for the Medium contaminated (1 to 3+/3) corresponding to an expected using probability of 2.5%.

C using for 0 false positive in healthy level and two samples less than expected is accepted for high levels ($2^+/2$ for the high and $3^+/5$ for the Medium Infected) and the number of positive samples for Medium contaminated is equal minimum $1^+/3$ corresponding to an expected using probability of 1%.

BMP (Below Minimum Performance) corresponds to a not expected result with a false positive in healthy or a false negative in high levels (0 or $1^+/2$) for the high levels; the number of positive samples for Medium contaminated is equal to $0^+/3$; the number of positive samples for Medium infected is <3+/5.

- **Rules of decision for identification part**

We adapted the tools for identification part. The column about healthy lot was not used in this case.

Rating	Nb of expected values		
	D. dipsaci	+	other
A	2	+	4
B	2	+	2
C	2	+	1
BMP	0	+	1

Statistical analysis of data

Raw data of all laboratories are given in appendix A.

Part 1: Extraction Part

2.2.1.1 Results for healthy, high levels (diagnostic specificity and sensitivity)

⇨ **Qualitative results**

Specificity and sensitivity

The analysis of results of 2 levels (Healthy and Highly contaminated "HC") 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°6.

The performance criteria are based on:

For the Specificity on N^+ = 2 samples
For the Sensitivity on N^- = 2 samples
For the Accuracy on N = 4 samples

Table n°6: Overview of qualitative results for each laboratory

Lab number	Healthy	High
Organizer	$0^+/2$	$2^+/2$
13	$1^+/2$	$2^+/2$
14	$0^+/2$	$2^+/2$
16	$0^+/2$	$2^+/2$
17	$0^+/2$	$2^+/2$
18	$0^+/2$	$1^+/2$
19	$0^+/2$	$2^+/2$
20	$1^+/2$	$1^+/2$
21	$0^+/2$	$1^+/2$

cell in grey correspond to lab results different from expected ones

Four laboratories (Lab 14; Lab 16; Lab 17; Lab 19) obtained the expected results at all levels of contamination.

3 laboratories (Lab 18; Lab 20; Lab 21): false negative results were observed in Highly contaminated
2 laboratories (Lab 13; Lab 20): false positive result was observed for the Healthy level.

Lab 20 obtain false positive and false negative results

Criteria of performance as specificity per lab are indicated in Table n°7.

Table n°7: Criteria of performance for each laboratory

Lab number	Sensitivity	Specificity	Accuracy
Organizer	100%	100%	100%
13	100%	50%	75%
14	100%	100%	100%
16	100%	100%	100%
17	100%	100%	100%
18	50%	100%	75%
19	100%	100%	100%
20	50%	50%	50%
21	50%	100%	75%

cell in grey correspond to lab results different from expected ones

Evaluation of performance criteria of participants:

4 laboratories of out 8 obtained 100% of sensitivity (no false negative), 100% of specificity (no false positive).

4 laboratories are non-conform on sensitivity or specificity:

Specificity 50% for the Lab 13 and Lab 20 with 1 out 2 negative results

Sensitivity: 50% for the Lab 18; Lab 20 and Lab 21 with 1 out of 2 positive results

2.2.1.2 Medium infected (MI)

In the pre-test, a count of the different nematodes was done. The results obtained show variability in the number of nematodes detected and the presence of other nematodes.

This lot is infected by alive *Ditylenchus dipsaci* and with other nematodes identified as *Aphelenchoides* sp.

During the process (pre-tests, homogeneity and stability tests) all samples were positive. The expected result is 5 positive samples. Results for each laboratory are given in table n°8.

Table n°8: analysis of results of laboratories

Lab number	N° of samples tested	Expected	Obtained	Conformity
Organizer	5	5	$5^{+}/5$	✓
13	5	5	$0^{+}/5$	✗
14	5	5	$4^{+}/5$	✗
16	5	5	$5^{+}/5$	✓
17	5	5	$2^{+}/5$	✗
18	5	5	$2^{+}/5$	✗
19	5	5	$4^{+}/5$	✗
20	5	5	$1^{+}/5$	✗
21	5	5	$1^{+}/5$	✗

1 laboratory obtained $5^{+}/5$ and 7 out of 8 laboratories obtained less than 5 positive samples for the Medium infected (MI) whose results are divided into 4 groups:

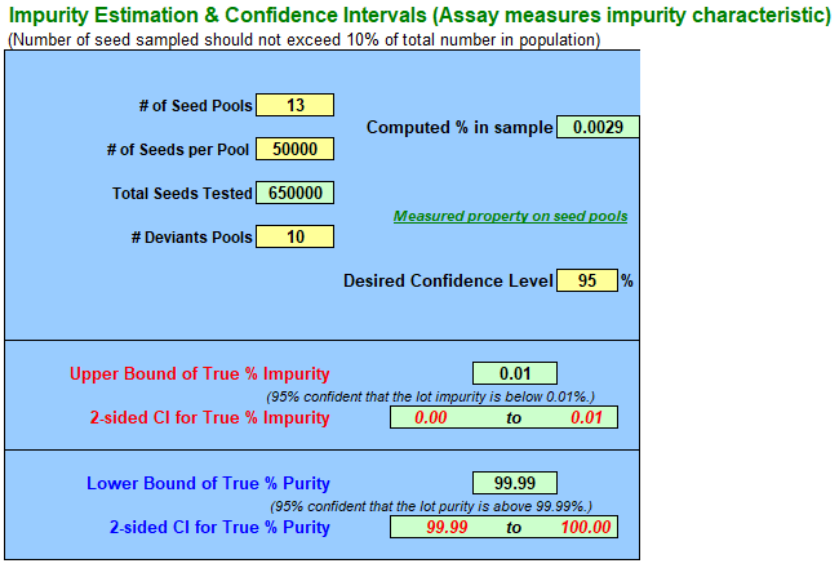
- $4^{+}/5$ for 2 laboratories (Lab 14; Lab 19)
- $2^{+}/5$ for 2 laboratories (Lab 17; Lab 18)
- $1^{+}/5$ for 2 laboratories (Lab 20; Lab21)
- $0^{+}/5$ for 1 laboratory (Lab13)

2.2.1.3 Medium contaminated (MC)

For the statistical analysis, we chose to use the results of the **homogeneity** and **stability** tests. This choice is linked to the fact of covering the whole organization and obtaining a maximum of values to allow a correct statistical analysis.

Results of both tests was used for the computation of probability to obtain contaminated samples out of tested samples. The percentage of contaminated obtained was 0.0029% (computed % in sample), corresponding to 10 positive samples out of 13 (Fig.3). Therefore, the probability at 5% to obtain positive samples was from 1 to 3 (Fig.4).

Figure 3: Results of medium level with the Seedcalc8 software.



Therefore, the probability at 5% to obtain positive samples was from 1 to 3 (Fig.4) out of 3 tested by laboratories. Results for each laboratory are given in table n°9.

Figure 4: Expected number of contaminated samples according to infection rate for 3 samples.

		k	Prob (%) of k positive out of n
# of seeds per sample (m)	50000	0	1.2906%
True contamination rate (π)	0.0029%	1	12.6345%
		2	41.2289%
Total # of samples (n)	3	3	44.8461%
		4	
		5	
		6	
		7	
		8	
		9	
		10	
		11	
		12	
		13	
		14	
		15	
		16	

k	Prob (%) of k positive out of n
0	1.2906%
1	12.6345%
2	41.2289%
3	44.8461%
4	#NOMBRE!
5	#NOMBRE!
6	#NOMBRE!
7	#NOMBRE!
8	#NOMBRE!
9	#NOMBRE!
10	#NOMBRE!
11	#NOMBRE!
12	#NOMBRE!
13	#NOMBRE!
14	#NOMBRE!
15	#NOMBRE!
16	#NOMBRE!

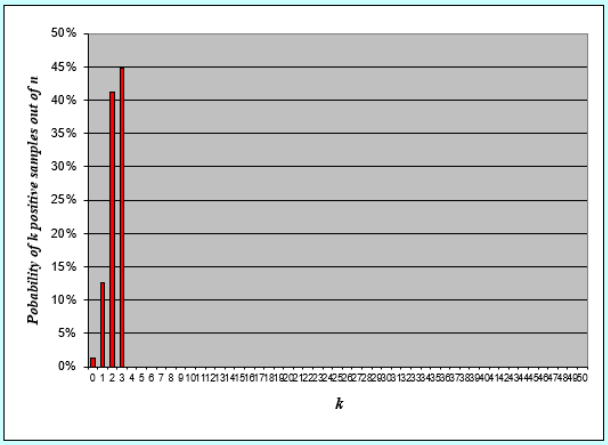
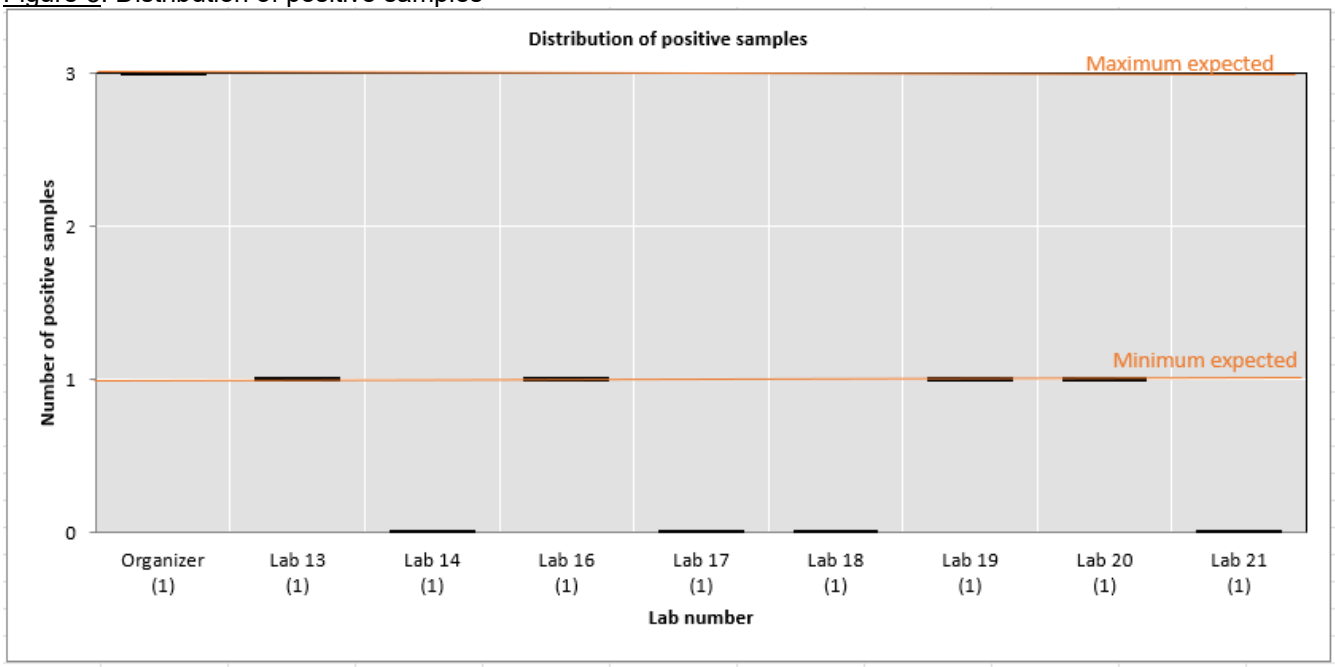


Table n°9: analysis of results of laboratories on MC samples

Lab number	N° of samples	Expected	Obtained	Conformity
Organizer	3	1 to 3	$3^{+}/3$	✓
13	3	1 to 3	$1^{+}/3$	✓
14	3	1 to 3	$0^{+}/3$	✗
16	3	1 to 3	$1^{+}/3$	✓
17	3	1 to 3	$0^{+}/3$	✗
18	3	1 to 3	$0^{+}/3$	✗
19	3	1 to 3	$1^{+}/3$	✓
20	3	1 to 3	$1^{+}/3$	✓
21	3	1 to 3	$0^{+}/3$	✗

The distribution of results is shown in Fig. 5.

Figure 5: Distribution of positive samples



Conclusion for medium level:
The results of medium level showed 3 groups of laboratories:

- 3 positive samples out of 3 for the organizer ‘s laboratory
- 1 positive sample out of 3 for 4 laboratories
- 0 positive sample out of 3 for 4 laboratories

50% of laboratories obtained the expected value range (from 1 to 3) and 50% of laboratories obtained 0 positive samples.

Identification part

- **Diagnostic specificity and sensitivity**

Raw data of all laboratories are given in Appendix B.

The expected results are indicated in table n°12. The number of correctly identified tubes for each laboratory are indicated in table n°13 and the level of performance criteria are indicated in table n°14.

The performance criteria were assessed separately as 2 entities: *Ditylenchus dipsaci* (2 tubes) and other nematodes (4 tubes) regrouping 2 tubes of *Ditylenchus destructor* +2 tubes of *Heterodera Schachtii*.

Table n°12: expected results of the part 2 (identification of nematodes)

Codification tube	Name of species	Expected results
A	*Ditylenchus destructor*	Other
B	*Heterodera schachtii*	Other
C	*Ditylenchus dipsaci*	*Ditylenchus dipsaci* (+ other)
D	*Heterodera Schachtii*	Other
E	*Ditylenchus destructor*	Other
F	*Ditylenchus dipsaci*	*Ditylenchus dipsaci* (+ other)

Table n°13: Overview of number of identified tubes for each laboratory

Lab number	Number of identified tubes as *D. dispsaci*			Number of identified tubes as Other		
	correct	+	incorrect	correct	+	incorrect
Organizer	**2**		**0**	4		0
13	2		0	4		0
14	2		0	2		2
16	2		0	2		2
17	2		0	4		0
18	1		1	3		1
19	2		0	3		1
20	1		1	1		3
21	1		1	4		0

cell in grey correspond to lab results different from expected ones

Evaluation of performance

The criteria of performance (sensitivity and specificity) per lab are indicated in Table 14.

Table n°14: Criteria of performance for each laboratory

Lab number	**Sensitivity**	**Specificity**	**Accuracy**
Organizer	100%	100%	100%
13	100%	100%	100%
14	100%	50%	75%
16	100%	50%	75%
17	100%	100%	100%

18	50%	75%	67%
19	100%	75%	88%
20	50%	25%	29%
21	50%	100%	84%

cell in grey correspond to lab results different from expected ones

2 laboratories obtained 100% for identification of *Ditylenchus dipsaci* and other.
1 laboratory indicated that they did not detect nematodes in A identified tubes. The results show a confusion between the different nematodes.

Conclusion:
The nematode *Ditylenchus dipsaci* was correctly identified by 2 laboratories out of 8.

Rating system

Part 1: Extraction part

The results are presented in table n° 11 and distribution of rating is presented figure 6.

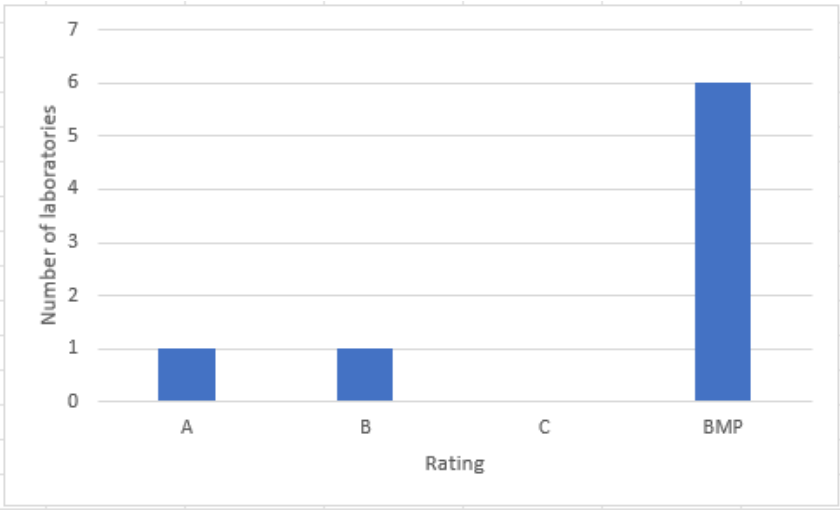
Table n°11: Computations of laboratories and rating

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

Minimum requirements for A rating :							
	Healthy lot		Highly lot			MC	Medium Infected =MI
Max # of pos reps	0	and	Min # of pos reps	2	and	Min prob for observing k	5.0%	5
Minimum requirements for B rating :							
	Healthy lot		Highly lot			MC	Medium Infected =MI
Max # of pos reps	0	and	Min # of pos reps	2	and	Min prob for observing k	2.5%	4
Minimum requirements for C rating :							
	Healthy lot		Highly lot			MC	Medium Infected =MI
Max # of pos reps	0	and	Min # of pos reps	2	and	Min prob for observing k	1.0%	3

						Medium level lot	
		Healthy lot	Highly lot		# of seeds/rep:	50000	Medium Infected = MI
					True cont. rate:	0.0029%	
					# of reps tested:	3	
Rating	Lab	# of pos reps	# of pos reps		# of pos reps (k)	Prob for observing k	# of pos reps
BMP	13	1	2		1	12.63%	0
BMP	14	0	2		0	1.29%	4
A	16	0	2		1	12.63%	5
BMP	17	0	2		0	1.29%	2
BMP	18	0	1		0	1.29%	2
B	19	0	2		1	12.63%	4
BMP	20	1	1		1	12.63%	1
BMP	21	0	1		0	1.29%	1

Figure n°6: Distribution of rating



The distribution of rating is spread over all letters.

1 laboratory obtained: an A rating
1 laboratory obtained: an B rating
6 laboratories obtained: an BMP rating

A and B ratings represents 25% of the laboratories.

The BMP rating is due:

- To a false negative or false positive result
- or/and a lower number of positive samples for MI (medium infected)

and figure 8 the distribution of rating.

Part 2: identification of nematodes

The results are presented in figure 8 and distribution of rating is presented figure 7.

Figure n° 7: Computations of laboratories and rating

Rating for qualitative SH P | Change any value in a yellow cell

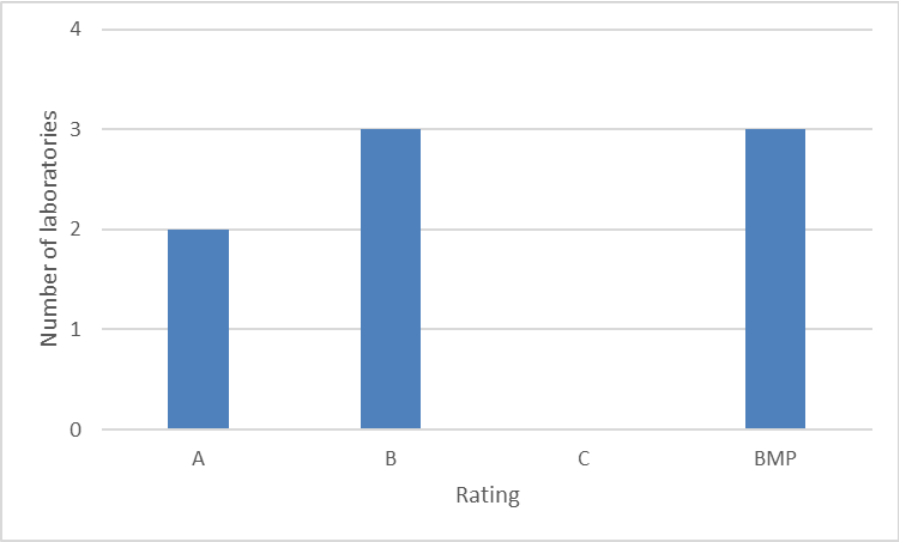
	Ditylenchus dipsaci		Other nematodes
Min # of pos reps:	2		4

	Ditylenchus dipsaci		Other nematodes
Min # of pos reps:	2		2

	Ditylenchus dipsaci		Other nematodes
Min # of pos reps:	2		1

Rating	Lab	*Ditylenchus dipsaci*		other nematodes
		# of pos reps		# of pos reps
A	13	2		4
B	14	2		2
B	16	2		2
A	17	2		4
BMP	18	1		3
B	19	2		3
BMP	20	1		1
BMP	21	1		4

Figure n°8: Distribution of rating



At the final, the rating is 3 ratings out of 4 (A; B and BMP). 2 laboratories achieved an A rating, three laboratories achieved an B rating, and 3 laboratories achieved an BMP rating.
The BMP rating is due a 1 out of 2 tubes are not identified as *Ditylenchus dipsaci* could not be identified by the labs for other nematodes.

CONCLUSION

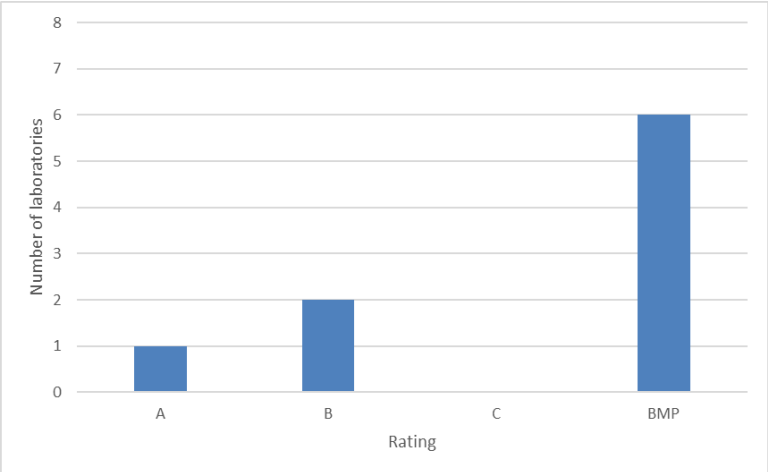
The table n°12 summary of the different results.

Table n°12: rating's summary

Lab number	Extraction	Identification	Final rating
13	BMP	A	BMP
14	BMP	B	BMP
16	A	B	B
17	BMP	A	BMP
18	BMP	BMP	BMP
19	B	B	B
20	BMP	BMP	BMP
21	BMP	BMP	BMP

The scores obtained on the extraction part are less good than for the identification. The number of BMP rating obtained is over for extraction than for identification parts. One laboratory obtained an A rating, 2 obtained an B rating and 6 obtained an BMP rating.

Figure n°9: Distribution of rating

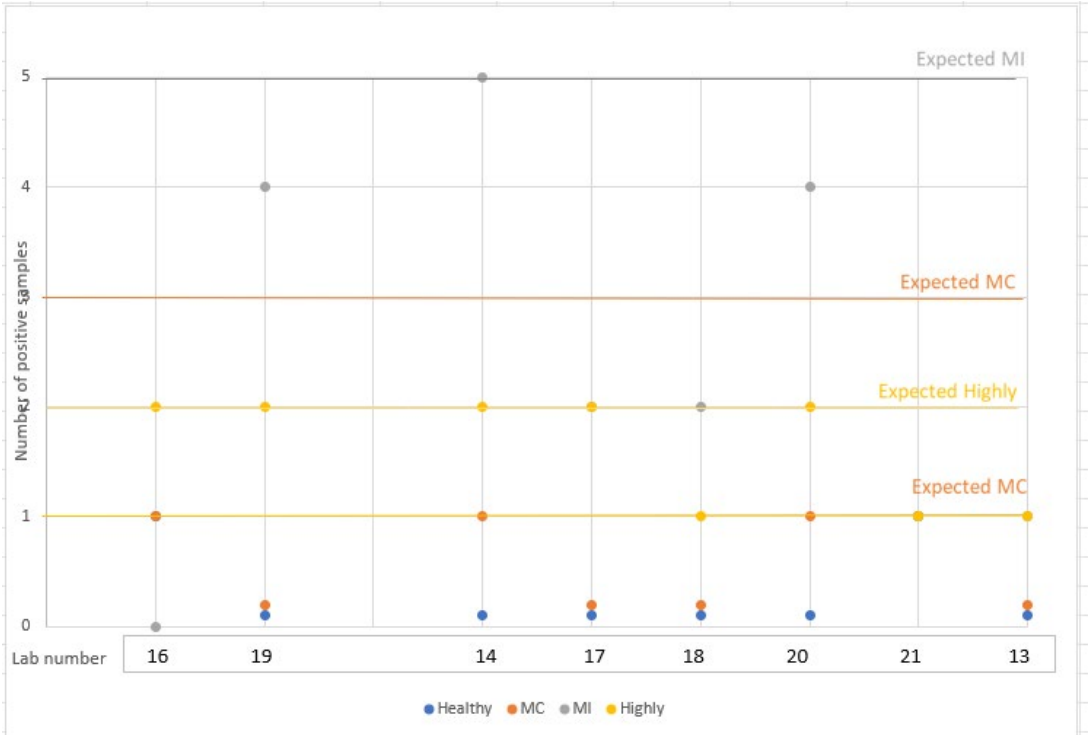


Part 1: extraction on seed samples

The healthy lot was tested negative during the process. It can't have had any cross-contamination. Positive results for healthy lot are therefore considered as false positive.

Is there a correlation in the extraction of nematodes for the expected positive lots?

Figure n°10: Distribution of samples by level



There was no correlation between results on the different positive samples. Labs 14 ,17,18 and 21 underestimate when the number of nematodes is not high which could show a limit of detection issue.

The highly contaminated lot was tested positive during the process, a negative result obtained is considered as false negative (Lab 18, 20 and 21)
The medium infected is a lot with different stages of nematode development (juveniles, adults) of *Ditylenchus dipsaci* and other nematodes which could have make it more difficult for some labs to identify.

In view of the results, we retested 3 samples from the MC lot to check that the nematodes had not been damaged by dehydration and were still recoverable during extraction.
The table presents the results.

Tableau n°13: results of the audit

Nb of repetition	Number of *Ditylenchus dipsaci*		Total
N°	Dead	Alive	
1	2	7	8
2	6	3	9
3	6	3	9

The method used for the contamination allows the extraction of the nematodes and we find living nematodes. We are above the LOD, with a recovery that is not of 100% as observed when the method was validated.
The detection rate of participants decreases highly when the lot is very low contaminated. It could indicate a limit of detection issue in some laboratories.

Part 2: identification of nematodes

The exercise of identification shows that the *Ditylenchus dipsaci* nematodes has been well identified by five laboratories (63% of identification rate). The other nematodes consisted of 2 types of nematodes *Heterodera schachtii* and *Ditylenchus destructor.*

Ditylenchus destructor is morphologically close to *Ditylenchus dipsaci*: the size is very similar, it's the same genus. The identification of the species is more difficult some criteria allow this differentiation as like the number of lines and the shape of the tail. But the participants did not make confusion between these 2 different species.

Criteria	*Ditylenchus dipsaci*	*Ditylenchus destructor*
Body length (um)	1000 – 1300	800-1400
Stylet length (um)	10-12	10 – 13
Number of lines	4	6
Shape of the tail	Short , knife or sword shaped	Blunt

Heterodera schachtii is a different genus. The criteria of identification is the size, it's due to the juvenile stage of nematodes.

The exercise of identification shows that the laboratories overall mastered the identification of nematodes *Ditylenchus dipsaci.*

Appendix A:
Raw data for extraction part

Lab number	Level of contamination	Number of samples	Expected result	Obtained results	Final result
13	Healthy	51	-	+	**$1^{+}/2$**
		80	-	-	
	Highly (HC)	43	+	+	$2^{+}/2$
		239	+	+	
	Medium infected (MI)	83	+	-	$0^{+}/5$
		90	+	-	
		129	+	-	
		167	+	-	
		189	+	-	
	Medium Contaminated (MC)	46	-/+	+	$1^{+}/3$
		104	-/+	-	
		171	-/+	-	
14	Healthy	37	-	-	$0^{+}/2$
		71	-	-	
	Highly (HC)	141	+	+	$2^{+}/2$
		238	+	+	
	Medium infected (MI)	6	+	+	$4^{+}/5$
		100	+	+	
		111	+	-	
		137	+	+	
		195	+	+	
	Medium Contaminated (MC)	59	-/+	-	$0^{+}/3$
		92	-/+	-	
		213	-/+	-	
16	Healthy	52	-	-	$0^{+}/2$
		210	-	-	
	Highly (HC)	2	+	+	$2^{+}/2$
		128	+	+	
	Medium infected (MI)	15	+	+	$5^{+}/5$
		81	+	+	
		101	+	+	
		205	+	+	
		218	+	+	
	Medium Contaminated (MC)	84	-/+	+	$1^{+}/3$
		86 ou 98	-/+	-	
		193	-/+	-	
17	Healthy	9	-	-	$0^{+}/2$
		94	-	-	
	Highly (HC)	140	+	+	$2^{+}/2$
		161	+	+	
	Medium infected (MI)	28	+	-	$2^{+}/5$
		160	+	+	
		206	+	-	
		215	+	+	
		227	+	-	
	Medium Contaminated (MC)	88	-/+	-	$0^{+}/3$
		120	-/+	-	
		203	-/+	-	
18	Healthy	173	-	-	$0^{+}/2$
		196	-	-	
	Highly (HC)	72	+	+	$1^{+}/2$
		97	+	-	
	Medium infected (MI)	47	+	-	$2^{+}/5$
		50	+	+	
		138	+	-	
		156	+	-	
		226	+	+	
	Medium Contaminated (MC)	98	-/+	-	$0^{+}/3$
		114	-/+	-	
		183	-/+	-	

Lab number	Level of contamination	Number of samples	Expected result	Obtained results	Final result
19	Healthy	85	-	-	$0^{+}/2$
		229	-	-	
	Highly (HC)	17	+	+	$2^{+}/2$
		41	+	+	
	Medium infected (MI)	13	+	-	$4^{+}/5$
		116	+	+	
		117	+	+	
		185	+	+	
		233	+	+	
	Medium Contaminated (MC)	30	-/+	-	$1^{+}/3$
		191	-/+	+	
		192	-/+	-	
20	Healthy	1	-	-	**$1^{+}/2$**
		154	-	+	
	Highly (HC)	112	+	-	**$1^{+}/2$**
		187	+	+	
	Medium infected (MI)	44	+	+	$1^{+}/5$
		105	+	-	
		108	+	-	
		134	+	+	
		214	+	-	
	Medium Contaminated (MC)	76	-/+	-	$1^{+}/3$
		77	-/+	+	
		147	-/+	-	
21	Healthy	329	-	-	0+/2
		349	-	-	
	Highly (HC)	321	+	-	**$1^{+}/2$**
		333	+	+	
	Medium infected (MI)	323	+	+	$1^{+}/5$
		326	+	-	
		337	+	-	
		338	+	-	
		339	+	-	
	Medium Contaminated (MC)	327	-/+	-	$0^{+}/3$
		340	-/+	-	
		351	-/+	-	

Appendix B: Raw data for identification part

Lab number	Identification of tubes	Expected values		Obtained values	
		Ditylenchus dipsaci	Others nematodes	*Ditylenchus dipsaci*	Others nematodes
		detected/ not detected	detected/ not detected	detected/ not detected	detected/ not detected
Lab 13	A	not detected	detected	not detected	detected
	B	not detected	detected	not detected	detected
	C	detected	detected	detected	not detected
	D	not detected	detected	not detected	detected
	E	not detected	detected	not detected	detected
	F	detected	detected	detected	not detected
Lab 14	A	not detected	detected	detected	not detected
	B	not detected	detected	not detected	detected
	C	detected	detected	detected	not detected
	D	not detected	detected	not detected	detected
	E	not detected	detected	detected	not detected
	F	detected	detected	detected	not detected
Lab 16	A	not detected	detected	detected	detected
	B	not detected	detected	detected	not detected
	C	detected	detected	detected	detected
	D	not detected	detected	detected	not detected
	E	not detected	detected	detected	detected
	F	detected	detected	detected	detected
Lab 17	A	not detected	detected	not detected	detected
	B	not detected	detected	not detected	detected
	C	detected	detected	detected	not detected
	D	not detected	detected	not detected	detected
	E	not detected	detected	not detected	detected
	F	detected	detected	detected	not detected
Lab 18	A	not detected	detected	not detected	detected
	B	not detected	detected	detected	not detected
	C	detected	detected	not detected	detected
	D	not detected	detected	not detected	detected
	E	not detected	detected	not detected	detected
	F	detected	detected	detected	not detected
Lab 19	A	not detected	detected	detected	not detected
	B	not detected	detected	not detected	detected
	C	detected	detected	detected	not detected
	D	not detected	detected	not detected	detected
	E	not detected	detected	not detected	detected
	F	detected	detected	detected	not detected
Lab 20	A	not detected	detected	not detected	not detected
	B	not detected	detected	detected	not detected
	C	detected	detected	detected	detected
	D	not detected	detected	detected	not detected
	E	not detected	detected	not detected	detected
	F	detected	detected	not detected	detected
Lab 21	A	not detected	detected	not detected	detected
	B	not detected	detected	not detected	detected
	C	detected	detected	not detected	detected
	D	not detected	detected	not detected	detected
	E	not detected	detected	not detected	detected
	F	detected	detected	detected	not detected