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Inter laboratory comparison (ILC) report*

ISTA PT23-SH 7-030

ISTA Proficiency test: Detection of *Acidovorax valerianellae* on Corn salad

*Original report signed and archived

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[1] : National Reference Laboratory (NRL) [2] : National Reference Laboratory (NRL)

Content table

Proficiency Test

1 PROFICIENCY TEST ORGANIZATION

The aim of this proficiency test was to verify the ability of laboratories to detect *Acidovorax valerianellae* (Av) in *Valerianella locusta* (corn salad) seeds. The schedule of this PT is presented in **Table 1**.

Table 1 : Schedule of the proficiency test

Sending of samples	6th of November 2023
Deadline to begin analysis	4th of December 2023
Deadline to send results	22nd of January 2024
Sending by GEVES of report and individual sheet	26th of April 2024

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

1.1 Type of results

The laboratories indicated qualitative results, the number of loci and the rate of saprophytic flora for each sample. They had to precise the method used.

1.2 Composition of the sample panel

14 samples of 5 000 corn salad seeds were sent to each laboratory. Three levels of contamination were represented in this panel, with a different number of samples per level as indicated in **Table 2**.

Table 2: Characteristics of samples in the panel

Level of contamination	Number of samples	Expected value for the detection of *Acidovorax valerianellae (*Av)
Healthy	3	not detected
Medium	8	detected
High	3	detected

1.3 Statistical tools

Results of participants will be compared to the expected results defined by the results of homogeneity test and/or stability test.

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.

1.3.1 Diagnostic sensitivity – specificity and accuracy

For homogeneous samples, the analysis was done by addition of the results of the 2 lots (healthy and highly infected level) according to the Standard NF EN ISO 16140 for qualitative results.

This norm gives us performance assessment criteria on diagnostic sensitivity, diagnostic specificity and accuracy calculated as in **Table 3** and **Table 4**.

Table 3: Evaluation criteria for sensitivity, specificity and accuracy

	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 negatives. ΣNA/(ΣNA+ΣPD)× 100.
Accuracy: (ΣNA+ΣPA)/ (ΣPA+ΣNA+ΣPD+ΣND) x100.

PA = positive agreement
ND = negative deviation
NA = negative agreement
PD = positive deviation

Table 4: 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

1.3.2 Seedcalc8 and Probability ISTA tools

Seedcalc8 program is a “probability tool for qualitative results” provided on the STATCOM webpage (tools), used to determine the % of infection of the seed.

1.3.3 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.

1.3.4 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.

1.4 Characterization of samples

1.4.1 Pre-test

Three seed lots were used in this PT:
Lot A: Healthy lot
Lot B: Medium infected lot with 50% of seeds from the A lot (2500 seeds per sample) and 50% of seeds from the C lot (2500 seeds per sample)
Lot C: Highly infected lot

3 subsamples of 5 000 seeds were tested for lots A and C and 8 subsamples were tested for the lot B, according to the ISTA 7-030 method (grow-out) on the 6th of June 2023. The results are given in **Table 5**. According to the results, lots A, B and C were accepted.

Table 5: Results of pre-test

Lot code	Contamination level	Obtained results		Decision
		Nb of loci	Result	
A	Healthy	0	$0^{+}/3$	Accepted
		0		
		0		
B	Medium	4	$8^{+}/8$	Accepted
		3		
		3		
		2		
		5		
		2		
		1		
		1		
C	High	6	$3^{+}/3$	Accepted
		5		
		5		

1.4.2 Homogeneity test

The homogeneity test was performed on the 26th of September 2023 after packaging and just before shipping of the seed samples to the participating laboratories. The method used to analyze the 30 samples was ISTA 7-030 method. 10 samples of 5 000 seeds for each level of contamination were tested for the detection of *Acidovorax valerianellae* (Av).

Expected results for each level are based on the pre-test results. The homogeneity test results are given in **Table 6**.

Table 6: Results of the homogeneity test

Lot code	Level of contamination	Expected results based on pre-test	Obtained results	Conformity
A	Healthy	$0^+/10$	$0^+/10$	Conform
B	Medium	$10^+/10$	$9^+/10$	Underestimate
C	High	$10^+/10$	$10^+/10$	Conform

Conclusion of homogeneity test:

Results obtained in the homogeneity test are in conformity with the pre-test results for the healthy and highly infected samples. For the medium level, the number of positive samples obtained was lower than the expected one (based on pre-test).

1.4.3 Stability Test

The stability test started on the 30th of January 2024. The method used to analyze the 30 samples was ISTA 7-030 method with confirmation by PCR (from ISTA method) for the doubtful symptoms.

Expected results are based on the homogeneity test results.

For the healthy level, no positive results were expected in the stability test ($0^+/15$) according to the results of the homogeneity test ($0^+/10$).

For the highly infected level, all results were expected positive in the stability test ($5^+/5$) according to the results of the homogeneity test ($10^+/10$).

For the medium level, the result of the homogeneity test was used for the computation of probability to obtain infected samples among tested samples. The percentage of infection obtained according to homogeneity test results was 0.05% (computed % in sample) corresponding to 9 positives out of 10 (**Figure 1**). Therefore, the probability at 5% to obtain positive samples was 8 to 10 out of 10 (**Figure 2**).

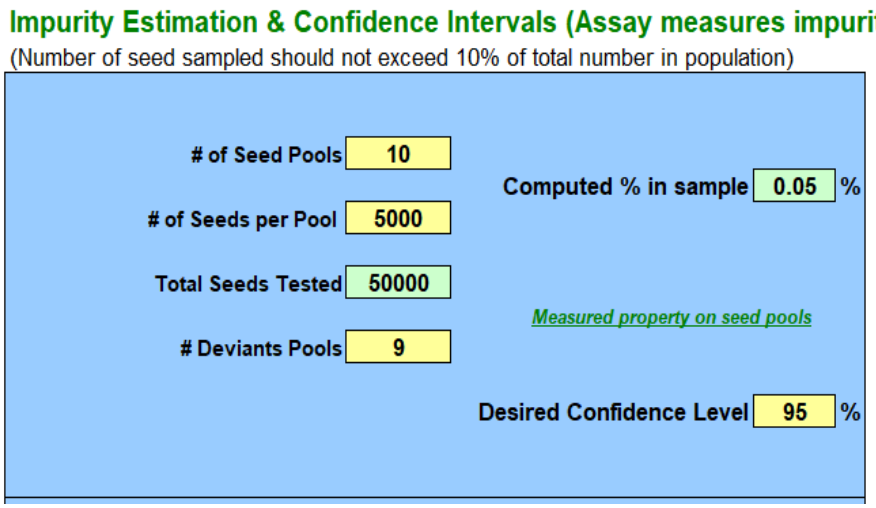

Figure 1: Percentage of infection of the medium infected sample according to homogeneity test results (Seedcalcl8)

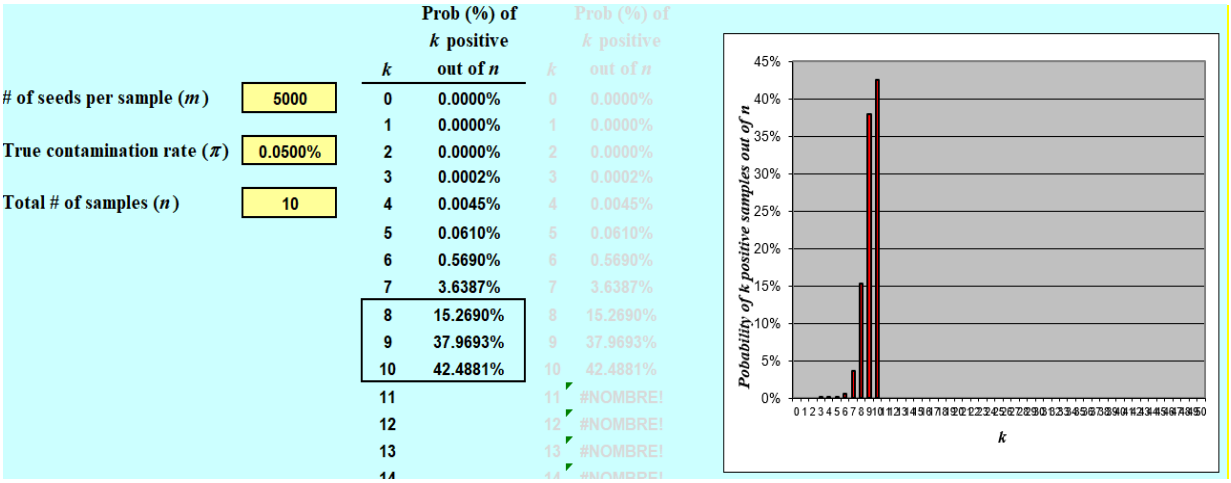

Figure 2: Expected number of infected samples among 10 medium infected samples according to the infection rate (Probability ISTA tools)

The results of the stability test are given in **Table 7**.

Table 7: Results of the stability test

Lot code	Level of contamination	Expected results based on homogeneity	Obtained results	Conformity
A	Healthy	$0^+/15$	$2^+/15$	overestimate
B	Medium	8^+ to $10^+/10$	$8^+/10$	Conform
C	High	$5^+/5$	$5^+/5$	Conform

Conclusion of stability test:

Results obtained in the stability test are in conformity with the homogeneity test results for medium and highly infected levels. For the healthy level, we observed 2 positive samples out of 15.

1.4.4 Conclusion of the characterization of samples

- Healthy level: $2^{+}/28$ were obtained which means that the contamination rate is very low (0.00148% with the confidence level to 95%, according to Seedcalcl8 tool).
- Medium level: $25^{+}/28$ were obtained which means that the contamination rate is 0.04% (confidence level to 95%, according to Seedcalcl8 tool).
- High level: we obtained $18^{+}/18$ all samples are positive.

These results will be taken into account in the statistical analysis of the participants' results.

2 ANALYSES OF PARTICIPANTS RESULTS

2.1 Statistical analysis of participants' results

The results of participating laboratories were compared to the expected results determined by the stability tests results. The raw data of all laboratories are given in appendix and the results of participating laboratories are given in **Table 8**.

Table 3: Overview of qualitative results obtained by each laboratory on the healthy, medium and highly infected samples

Lab number	Healthy	Medium	High
01	$1^{+}/3$	$7^{+}/8$	$3^{+}/3$
09	$1^{+}/3$	$6^{+}/8$	$3^{+}/3$

2.1.1 Diagnostic sensitivity-specificity and accuracy

The obtained results for healthy and highly infected lots during the stability test does not allow the test to be used for the 3 criteria (sensitivity - specificity and accuracy). In this case, it is not possible to use the healthy lot to determine the value of specificity because positives results were found during the stability test in this lot.

The statistical test is performed only on highly infected level to define the value of sensitivity as described in the part 1.3.1 ($\Sigma PA/(\Sigma PA+\Sigma ND)x100$). All infected samples are positive, no false negative results have been obtained.

The diagnostic sensitivity was calculated with the results obtained on the 3 highly infected samples (expected to be positive). Results of participating laboratories and the percentage of sensitivity for each laboratory are presented in **Table 9**.

Table 9: Overview of qualitative results on highly infected samples and the percentage of sensitivity for each laboratory

Lab number	High	Sensitivity

01	$3^+/3$	100%
09	$3^+/3$	100%

All laboratories obtained 100% of sensitivity (no false negative).

The Seedcalc8 and Probability ISTA tools (part 1.3.2) and Probability of k positive samples out of n (part 1.3.3) will be used for healthy and medium levels.

2.1.2 Medium infected level

For the medium level, the result of the stability test was used for the computation of probability to obtain infected samples among tested samples. The percentage of infection obtained according to stability test results was 0.03% (computed % in sample) corresponding to 8 positives out of 10 (**Figure 3**). The expected positive samples tested by laboratories was: 4 to 8 out of 8 for an acceptation at 5% (**Figure 4**).

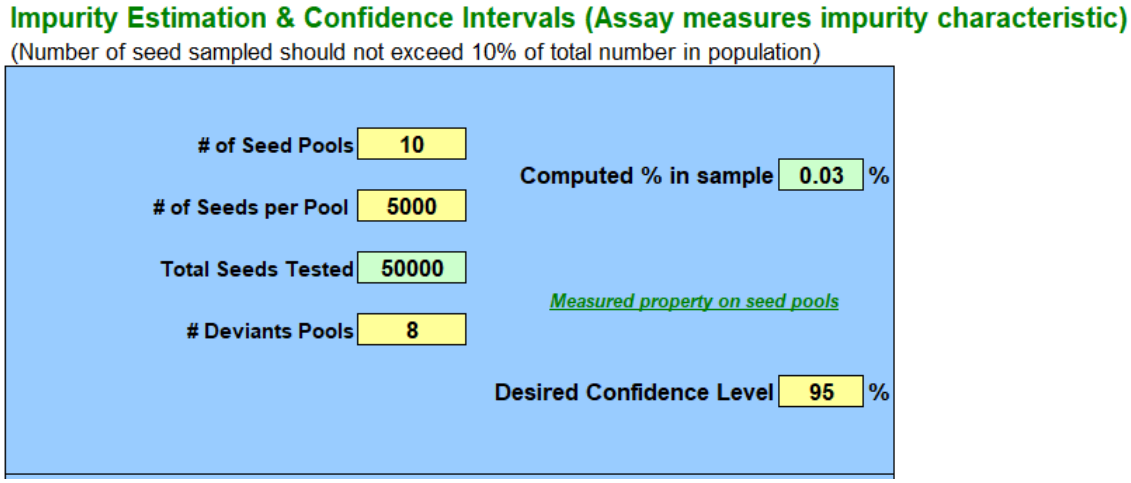

Figure 3: Percentage of infection of the medium infected sample according to stability test results (Seedcalcl8)

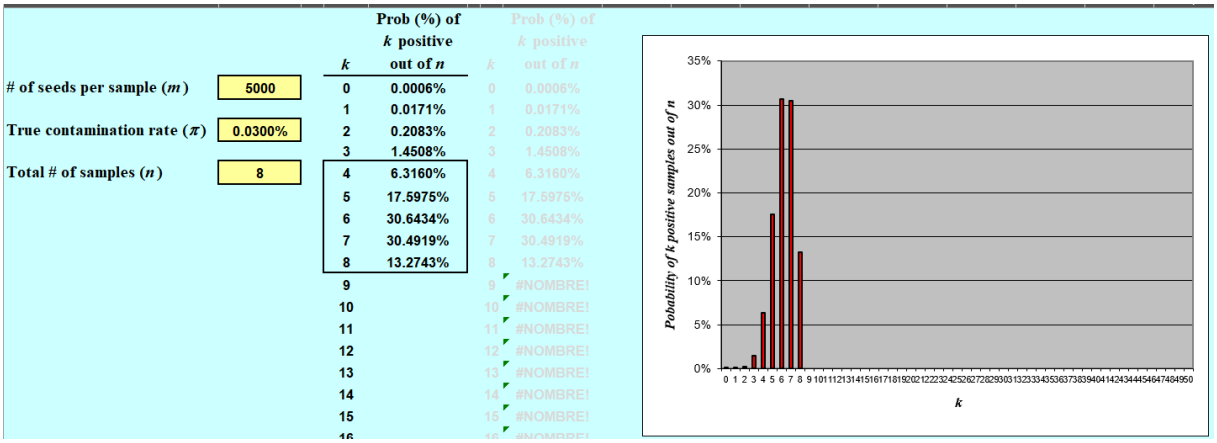

Figure 4: Expected number of infected samples among 10 medium infected samples according to the infection rate (Probability ISTA tools).

Results for each laboratory are given in **Table 10**.

Table 10: Analysis of results of laboratories for medium infected level

Lab number	Number of samples tested	Number of positive samples expected	Number of positive samples obtained	Conformity
02	8	4 to 8⁺	7	✓
09	8	4 to 8⁺	6	✓

Conclusion: all laboratories are conform with expected values.

2.1.3 Healthy level

Results of stability test were used for the computation of probability to obtain infected samples out of tested samples. The percentage of infection obtained was 0.0029% (computed % in sample), corresponding to 2 positive samples out of 15 (**Figure 5**). The expected positive samples tested by laboratories was 0 to 1 (**Figure 6**) out of 3 for an acceptation at 5%.

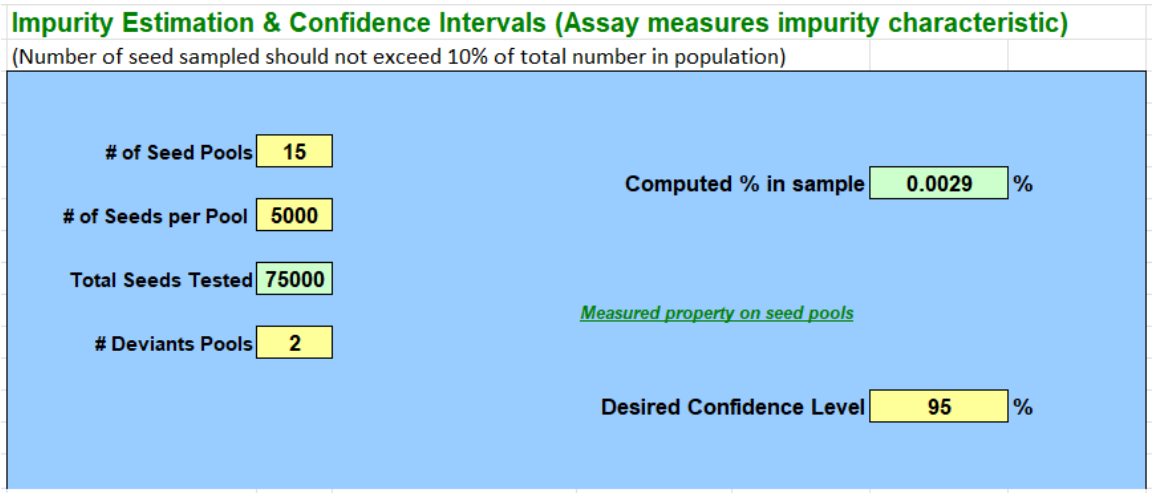

Figure 5: Percentage of infection of the healthy sample according to stability test results

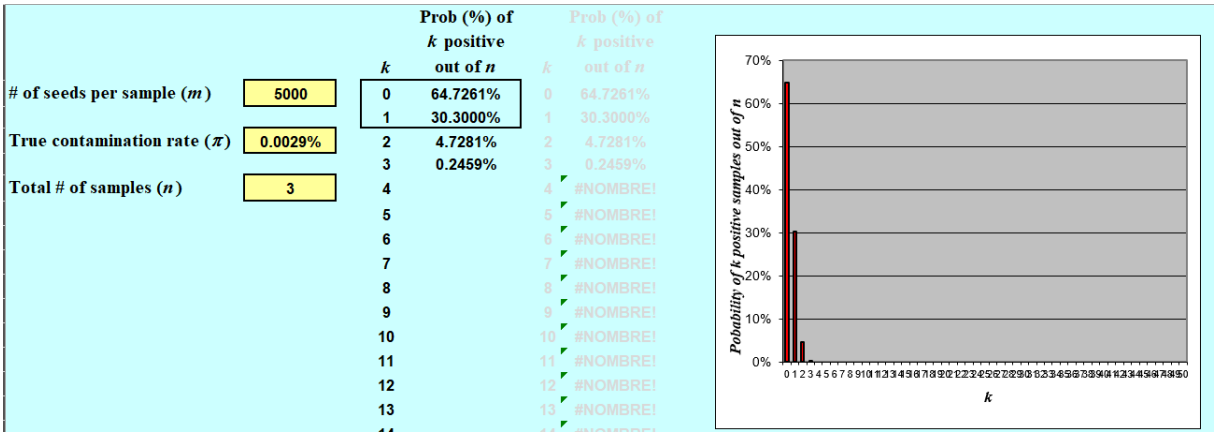

Figure 6: Expected number of infected samples among 3 healthy samples according to the infection rate (Probability ISTA tools)

Results for each laboratory are given in **Table 11**.

Table 11: Analysis of results of laboratories for healthy level

Lab number	Number of samples tested	Number of positive samples expected	Number of positive samples obtained	Conformity
01	3	0 to 1^+	1	✓
09			1	✓

Conclusion for healthy level: All laboratories obtain the expected value range.

2.1.4 Rating system

The decision rule is as follows:

- A corresponds to an expected result using a probability of 5% for the healthy and medium infected levels and no false negative in high level.
- B using a probability of 2,5% for the healthy and medium infected levels and no false negative in high level.
- C using a probability under 1% for the healthy and medium infected levels and no false negative in high level.
- BMP (Below Minimum Performance) corresponds to a false negative result in high level or < 1% for healthy and medium infected levels.

The calculation of the rating for each laboratory is presented (**Figure 12)**.

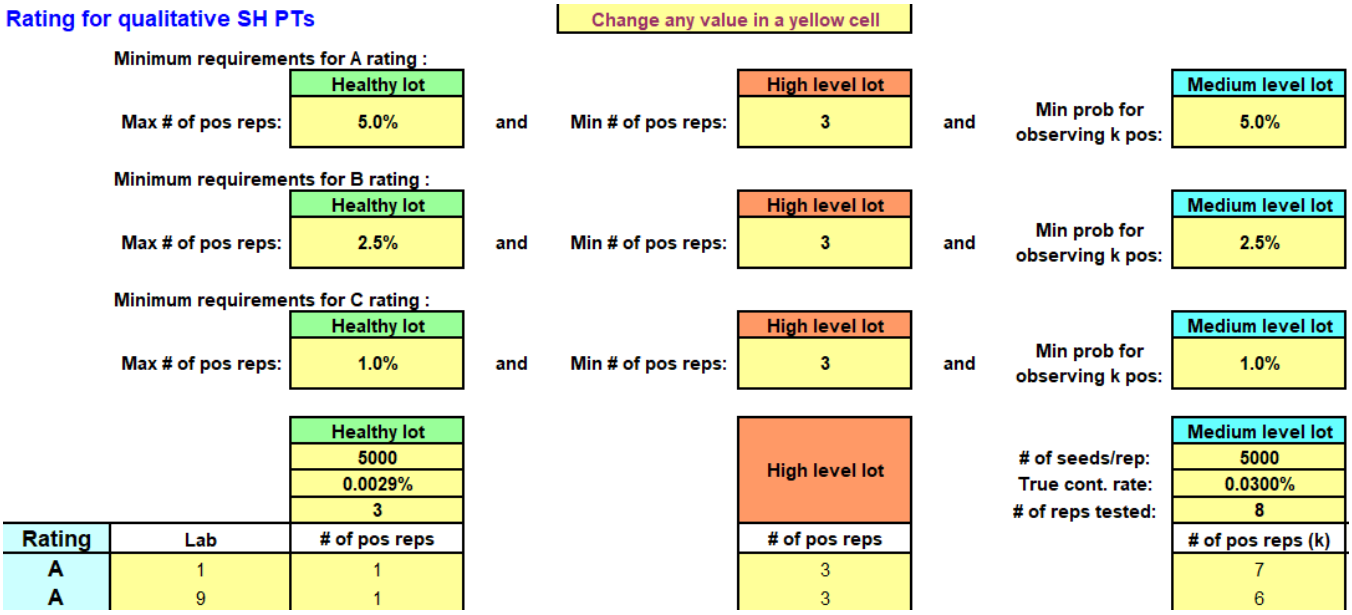

Figure 12: Computation of ratings for each laboratory

2.2 ***Method declared by participants***

Based on the indications provided by the laboratories, one method was used: grow-out followed by PCR test.

3 CONCLUSIONS

For this proficiency test, the A rating represents 100% of the laboratories. The number of laboratories with each rating is presented in the **Figure 13**.

Figure 13: Number of laboratories with each rating

Appendix:

Lab number	Level of contamination	Number of samples	Obtained results	
Lab 01	Healthy	71	-	1+/3
	Healthy	126	+	
	Healthy	127	-	
	High	80	+	3^{+}/3
	High	97	+	
	High	134	+	
	Medium	12	+	7^{+}/8
	Medium	15	+	
	Medium	42	+	
	Medium	52	+	
	Medium	54	+	
	Medium	56	-	
	Medium	98	+	
	Medium	124	+	
Lab 09	Healthy	47	-	1^{+}/3
	Healthy	111	+	
	Healthy	132	-	
	High	3	+	3^{+}/3
	High	83	+	
	High	93	+	
	Medium	28	+	6^{+}/8
	Medium	31	-	
	Medium	44	+	
	Medium	45	-	
	Medium	103	+	
	Medium	123	+	
	Medium	135	+	
	Medium	138	+	