

# Proficiency test (ILC) report* 

Organized by the National Seed Testing Station (SNES) of GEVES

Final

## 2019-ISTA-FLAX

ISTA Proficiency test: Detection of 3 pathogens (Alternaria linicola, Botrytis cinerea, Colletotrichum lini,) in Linum usitatissimum (flax, linseed) seeds

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## Detection of 3 pathogens in flax seeds

## 1 PROFICIENCY TEST ORGANIZATION

The aim of this Proficiency Test was to verify the ability of laboratories to detect and identify 3 pathogens (Alternaria linicola, Botrytis cinerea, Colletotrichum lini) in Linum usitatissimum (Flax, linseed) seeds.

Schedule

| Sending of samples | $24^{\text {th }}$ of June |
| :--- | :---: |
| Deadline to send results | $2^{\text {nd }}$ of August |
| Sending by GEVES of global report and <br> individualized letters | $31^{\text {st }}$ of March |

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

On 21 participants registered for the proficiency test:
-10 were accredited for 7-007 method.
-11 were not accredited for 7-007 method.

### 1.1 Notation of results

The laboratories indicated:

- a qualitative result (positive, negative)
- a quantitative result for each sample (\% for each pathogen)


### 1.2 Composition of the sample panel

9 samples of 400 flax seeds have been sent to each participant with 3 replicates for each level of contamination (table $\mathrm{n}^{\circ} 1$ ).

Table n ${ }^{\circ}$ 1: Characteristics of samples

| Number of <br> samples | Level of <br> contamination | Qualitative expected <br> value |
| :---: | :---: | :---: |
| 3 | Healthy | Negative |
| 3 | Medium | Positive |
| 3 | High | Positive |

Each sample was sent in a sealed bag.

### 1.3 Pretest

The objective was to obtain a contamination rate close to $5 \%$ for the medium level and $10 \%$ for the high level for each pathogen. Four seeds lots have been tested in 10 subsamples of 400 seeds by ISTA method 7-007. The results of pre-tests are indicated in table $\mathrm{n}^{\circ} 2$.

Table $n^{\circ} 2$ : results of pretests

| Lot code | Pathogen | $\%$ of <br> contamination | Comments |
| :---: | :--- | :---: | :--- |
| A | Colletotrichum linicola | 0 |  |
|  | Alternaria linicola | 30 |  |
| Botrytis cinerea | 0 |  |  |
| C | Colletotrichum linicola | 10 |  |
|  | Alternaria linicola | 0 |  |
|  | Botrytis cinerea | 0 |  |
| S | Colletotrichum linicola | 0 |  |
|  | Alternaria linicola | 0 |  |
| H | Botrytis cinerea | 0 |  |
|  | Colletotrichum linicola | 0 | Presence of saprophytes that made the |
|  | Alternaria linicola | 0 | notation difficult |

The lot H was cancelled due to the presence of saprophytes
3 levels of contamination: healthy, medium and high levels were created. The both levels (medium and high) were obtained by blending naturally contaminated lots and spiking with artificially contaminated seeds. The composition of samples was indicated in table $n^{\circ} 3$.

Table $n^{\circ} 3$ : composition of samples

| Codification of <br> lot | Pathogen | Type of contamination |
| :---: | :--- | :---: |
| C | Colletotrichum lini | natural |
| E |  | artificial |
| A | Alternaria linicola | natural |
| D |  |  |
| B | Botrytis cinerea | artificial |
| S | Healthy |  |

The medium level was obtained by spiking with artificially contaminated seeds for Botrytis cinerea and naturally contaminated seeds for Alternaria linicola and Colletotrichum lini.

The high level was obtained by blending the artificially contaminated seeds pathogen/pathogen in healthy seeds.

### 1.4 Homogeneity Test

The statistical analysis was done with the homogeneity test tool (Hampel's method) for each pathogen Homogeneity test was done after packaging and just before sending. 10 extra samples of 400 seeds representing each contamination level were tested. The samples have been tested the 12 th of June.

The raw data are given in Appendix A.

### 1.4.1 Healthy

All samples were free of all pathogens.

### 1.4.2 Medium level

The results for homogeneity test for the medium level are given in figure 1.
Figure 1: Homogeneity test results for medium level.

| Pathogen |  |  | Ham | mpel |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | MS Excel | Hampels Outlier | Test Example |  |  |  |
|  | A linicola |  |  |  |  |  |
|  | Lab | Lab Values (Xi) | \| Xi - M ${ }^{\text {l }}$ | Status |  |  |
|  | 1. | 1.4 .00 | 1.000 | OK | Median (M): | 5.000 |
|  | 2 | 2.3 .75 | 1.250 | OK | MAD: | 0.795 |
|  | 3 | 3.3 .25 | 1.750 | OK | $5.2 \times \mathrm{MAD}$ | 4.134 |
| Alternaria linicola | 4. | . 5.00 | 0.000 | OK |  |  |
|  | 5 | 5 $\quad 5.00$ | 0.000 | OK |  |  |
|  | 6 | 6 6.50 | 1.500 | OK |  |  |
|  | 7 | 7 5.00 | 0.000 | OK |  |  |
|  | 8 | 3 $\quad 5.00$ | 0.000 | OK |  |  |
|  | 9 | 9 5.84 | 0.840 |  |  |  |
|  | 10 | 5.75 | 0.750 |  |  |  |
|  |  |  |  |  |  |  |
|  | MS Excel Ha | ampels Outlier | Test Examp |  |  |  |
|  | Colleto |  |  |  |  |  |
|  | Lab L | Lab Values ( $\mathrm{Xi}^{\text {i }}$ ) | $\mid \mathrm{Xi}$-M\| | Status |  |  |
|  | 1. | 6.00 | 0.460 | OK | Median (M): | 5.540 |
|  | 2 | 6.00 | 0.460 | OK | MAD: | 1.000 |
|  | 3 | 4.25 | 1.290 | OK | $5.2 \times \mathrm{MAD}$ | 5.200 |
| Colletotrichum lini | 4 | 8.50 | 2.960 |  |  |  |
| Colletotrichum lini | 5 | 4.25 | 1.290 | OK |  |  |
|  | 6 | 7.00 | 1.460 | OK |  |  |
|  | 7 | 3.75 | 1.790 | OK |  |  |
|  | 8 | 5.00 | 0.540 | OK |  |  |
|  | 9 | 5.08 | 0.460 | OK |  |  |
|  | 10 | 6.25 | 0.710 |  |  |  |
| Botrytis cinerea | MS Excel Hampels Outlier Test Example |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  | Lab | Lab Values ( Xi ) | $\|\mathrm{Xi}-\mathrm{M}\|$ | Status |  |  |
|  | 1. | 4.75 | 0.920 | OK | Median (M): | 5.670 |
|  | 2 | 6.00 | 0.330 | OX | MAD: | 0.330 |
|  | 3. | 7.00 | 1.330 |  | $5.2 \times \mathrm{MAD}$ | 1.716 |
|  | 4. | 6.00 | 0.330 |  |  |  |
|  | 5 | 6.00 | 0.330 |  |  |  |
|  | 6 | 5.50 | 0.170 | OK |  |  |
|  | 7 | 4.00 | 1.670 | OK |  |  |
|  | 8 | 5.25 | 0.420 |  |  |  |
|  | 9 | 5.84 | 0.170 | OK |  |  |
|  | 10 | 5.50 | 0.170 | OK |  |  |
|  | 11 |  |  |  |  |  |

## - Repartition of pathogens

Results of the ten extra samples given in figure 2 show the dispersion against the mean.
Figure ${ }^{\circ}$ 2: Homogeneity test results, repartition against the mean


## - 1.4.3 High level

The results for homogeneity test for the high level are given in figure 3 .
Figure 3: Homogeneity test results for high level.

| Pathogen | Hampel |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Alternaria linicola | MS Excel Hampels Outlier Test Example |  |  |  |  |  |  |
|  | Lab | Lab Values (Xi) | $\mid \mathrm{Xi}$ - M \| | Status |  |  |  |
|  | 1. | 9.00 | 1.250 | OK | Median (M): | 10.250 |  |
|  | 2 | 10.25 | 0.000 | OK | MAD: | 0.625 |  |
|  | 3 | 10.00 | 0.250 | OK | 5.2 X MAD | 3.250 |  |
|  | 4 | 10.75 | 0.500 | OK |  |  |  |
|  | 5 | 10.25 | 0.000 | OK |  |  |  |
|  | 6 | 9.50 | 0.750 | OK |  |  |  |
|  | 7 | 11.75 | 1.500 | OK |  |  |  |
|  | 8 | 11.50 | 1.250 | OK |  |  |  |
|  | 9 | 10.50 | 0.250 | OK |  |  |  |
|  | 10 | 9.25 | 1.000 | OK |  |  |  |


| Colletotrichum lini | MS Excel Hampels Outlier Test Example |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Lab | Lab Values ( Xi ) | $\|\mathrm{Xi}-\mathrm{M}\|$ | Status |  |  |
|  | 1 | 13.50 | 0.375 | OK | Median (M): | 13.875 |
|  | 2 | 13.75 | 0.125 | OK | MAD: | 0.875 |
|  | 3 | 14.75 | 0.875 | OK | 5.2 X MAD | 4.550 |
|  | 4 | 15.75 | 1.875 | OK |  |  |
|  | 5 | 16.00 | 2.125 | OK |  |  |
|  | 6 | 12.75 | 1.125 | OK |  |  |
|  | 7 | 10.50 | 3.375 | OK |  |  |
|  | 8 | 13.00 | 0.875 | OK |  |  |
|  | 9 | 14.75 | 0.875 | OK |  |  |
|  | 10 | 14.00 | 0.125 | OK |  |  |
|  | MS Excel H | Hampels Outlier | Test Exam |  |  |  |
|  |  |  |  |  |  |  |
|  | Lab | Lab Values (Xi) | \| Xi - M| | Status |  |  |
|  | 1 | 10.25 | 0.375 | OK | Median (M): | 10.625 |
|  | 2 | 9.00 | 1.625 | OK | MAD: | 1.000 |
|  | 3 | 10.00 | 0.625 | OK | 5.2 X MAD | 5.200 |
| Botrytis cinerea | 4. | 12.00 | 1.375 | OK |  |  |
|  | 5 | 11.25 | 0.625 | OK |  |  |
|  | 6 | 11.75 | 1.125 | OK |  |  |
|  | 7 | 13.00 | 2.375 | OK |  |  |
|  | 8 | 9.50 | 1.125 | OK |  |  |
|  | 9 | 11.00 | 0.375 | OK |  |  |
|  | 10 | 9.75 | 0.875 | OK |  |  |

## - Repartition of pathogen

Results of the ten extra samples given in figure 4 show the dispersion against the mean.
Figure 4: Homogeneity test results, repartition against the mean


## Conclusion

The samples were homogeneous:

- For healthy level, we obtained 0 positive samples. No false positive obtained.
- For medium and high levels, the samples were homogeneous for each pathogen. The average obtained was close to the expected percentage.


### 1.5 Stability Test

The stability testing was conducted after all laboratories started testing. The stability test has been started the $15^{\text {th }}$ of April.
5 extra samples of 400 seeds were tested for each level. The raw data are given in Appendix A.
For the healthy lot, all samples were negative.
The comparison between homogeneity and stability tests for each pathogen is indicated in table $\mathrm{n}^{\circ} 4$ and figure 5.

Table n ${ }^{\circ} 4$ : Comparison between homogeneity and stability results

| Level of contamination | Alternaria linicola (\%) |  |  | Botrytis cinerea (\%) |  |  | Colletotrichum lini (\%) |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Homogeneity | Stability | deviation | Homogeneity | Stability | deviation | Homogeneity | Stability | deviation |
| Medium | 4.91 | 5.26 | 0.35 | 5.28 | 4.96 | -0.32 | 5.91 | 5.76 | -0.15 |
| High | 10.28 | 10.00 | -0.28 | 10.75 | 11.15 | 0.40 | 13.88 | 14.20 | 0.32 |

"-"indicated a slight decrease between homogeneity and stability

Figure 5: Comparison between homogeneity and stability tests


## Conclusion

Stability of the lots has been confirmed:
-healthy level was negative
-medium level: the obtained results (\%) were similar than homogeneity tests (deviation between 0.04 and $0.35 \%$ )

- high level: the obtained results (\%) were similar than homogeneity tests for the all pathogens. (deviation between -0.28 and $0.4 \%$ ).


### 1.6 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 and stability tests.

## 2 PROFICIENCY TEST RESULTS

### 2.1 Qualitative results

### 2.1.1 Statistical tools

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 $+\quad$ (contaminated <br> sample) | expected result - (healthy sample) |
| :--- | :--- | :--- |
| Obtained result + | positive agreement $+/+(\mathrm{PA})$ | positive deviation -/+ (PD) |
| Obtained result - | negative deviation $+/-$ (ND) | negative agreement $-/-$ (NA) |

Sensitivity: Percentage of samples correctly identified as positives. $\Sigma \mathrm{PA} /(\Sigma \mathrm{PA}+\Sigma \mathrm{ND}) \mathrm{x} 100$.
Specificity: Percentage of samples correctly identified as being negative. $\Sigma \mathrm{NA} /(\Sigma \mathrm{NA}+\Sigma \mathrm{PD}) \times 100$. Accuracy: $(\Sigma N A+\Sigma \mathrm{PA}) /(\Sigma \mathrm{PA}+\Sigma \mathrm{NA}+\Sigma \mathrm{PD}+\Sigma \mathrm{ND}) \mathrm{x} 100$.
$\mathrm{PA}=$ positive agreement
ND = negative deviation
$\mathrm{NA}=$ negative agreement
$\mathrm{PD}=$ positive deviation
$\mathrm{N}=$ total number of possible agreements
Conformity of results:

| Performance criteria | Level to obtain |
| :--- | :--- |
| Sensitivity | $100 \%:$ all contaminated samples are positive; no false negative <br> results have been obtained |


| Specificity | $100 \%:$ all healthy samples are negative; no false positive results <br> have been obtained |
| :--- | :--- |
| Accuracy | Synthesis of the two performance criteria. So, no false positive or <br> negative results have been obtained |

The analysis of the results for a participating laboratory led to a declaration of conformity or nonconformity of the results in an individual sheet.

- "conform": obtained results correspond to expected results.
- "not conform": obtained results do not correspond to expected results.


### 2.1.2 Statistical analysis of data

Results and performance criteria are given in table $n^{\circ} 5$ and table $n^{\circ} 6$.
Table n ${ }^{\circ}$ 5: Overview of qualitative results for each laboratory on the 3 levels

| Lab number | Participation | Alternaria linicola |  |  | Botrytis cinerea |  |  | Colletotrichum lini |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Healthy | Medium | High | Healthy | Medium | High | Healthy | Medium | High |
| 01 | obligatory | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 02 | obligatory | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 03 | obligatory | $0^{+} / 3$ | $0^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 04 | obligatory | $0^{+} / 3$ | $2^{+} / 3$ | $3+/ 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 05 | obligatory | $0^{+} / 3$ | 0+/3 | $0^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 06 | obligatory | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 07 | obligatory | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 08 | obligatory | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 09 | obligatory | $2^{+} / 3$ | $2^{+} / 3$ | $2^{+} / 3$ | $2^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 11 | obligatory | $0^{+} / 3$ | $3^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 12 | voluntary | 3+/3 | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ |
| 13 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $2^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 14 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 15 | voluntary | $0^{+} / 3$ | $2^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 16 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $1^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 17 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 19 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 20 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $1^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 22 | voluntary | $0^{+} / 3$ | $2^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 24 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $2^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |
| 25 | voluntary | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ | $0^{+} / 3$ | $2^{+} / 3$ | $2^{+} / 3$ | $0^{+} / 3$ | $3^{+} / 3$ | $3^{+} / 3$ |

Table $n^{\circ} 6$ : Criteria of performance for each laboratory

| Lab number | Participation | Alternaria linicola |  |  | Botrytis cinerea |  |  | Colletotrichum lini |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Sensitivity | Specificity | Accuracy | Sensitivity | Specificity | Accuracy | Sensitivity | Specificity | Accuracy |
| 01 | obligatory | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 02 | obligatory | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 03 | obligatory | 33\% | 100\% | 56\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 04 | obligatory | 83\% | 100\% | 89\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 05 | obligatory | 0\% | 100\% | 33\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 06 | obligatory | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 07 | obligatory | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 08 | obligatory | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 09 | obligatory | 67\% | 33\% | 56\% | 100\% | 33\% | 78\% | 100\% | 100\% | 100\% |
| 11 | obligatory | 83\% | 100\% | 89\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 12 | voluntary | 100\% | 0\% | 67\% | 0\% | 100\% | 33\% | 0\% | 100\% | 33\% |
| 13 | voluntary | 100\% | 100\% | 100\% | 67\% | 100\% | 78\% | 100\% | 100\% | 100\% |
| 14 | voluntary | 50\% | 100\% | 67\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 15 | voluntary | 83\% | 100\% | 89\% | 0\% | 100\% | 33\% | 100\% | 100\% | 100\% |
| 16 | voluntary | 67\% | 100\% | 78\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 17 | voluntary | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 19 | voluntary | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% | 100\% |
| 20 | voluntary | 83\% | 100\% | 89\% | 67\% | 100\% | 78\% | 100\% | 100\% | 100\% |
| 22 | voluntary | 67\% | 100\% | 78\% | 83\% | 100\% | 89\% | 100\% | 100\% | 100\% |
| 24 | voluntary | 100\% | 100\% | 100\% | 67\% | 100\% | 78\% | 100\% | 100\% | 100\% |
| 25 | voluntary | 100\% | 100\% | 100\% | 67\% | 100\% | 78\% | 100\% | 100\% | 100\% |

7 out of 21 laboratories obtained the expected result for all pathogens and obtained $100 \%$ of sensitivity (no false negative) and $100 \%$ of specificity (no false positive).

14 out of 21 laboratories obtained false positive and/or false negative results:

- false positive:
- Alternaria linicola: 2 laboratories (Lab 09; Lab 12)
- Botrytis cinerea: 1 laboratory (Lab 09)
- false negative:
- Alternaria linicola:
- Medium : 6 laboratories (Lab 03;04;05;09;15;22)
- High :8 laboratories (Lab 03;05;09;11;14;16;20;22)
- Botrytis cinerea :
- Medium : 5 laboratories (Lab 12;13;15;24,25)
- High : 7 laboratories (Lab 12;13;15;20;22;24,25)
- Colletotrichum lini
- Medium : 1 laboratory (Lab 12)
- High : 1 laboratory (Lab 12)

We observed that Colletotrichum lini was better detected than the other two pathogens. The rate classification of detection whatever the level is:

Colletotrichum lini > Botrytis cinerea $>$ Alternaria lincola

### 2.1.3 Rating: Z-score-computations and rating system

## 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. We use a qualitative rating system.

## Rules of decision

For use the qualitative rating, medium and high levels have been grouped.
A correspond to no false positive result for the healthy level and no false negative result for medium and high levels grouped (6 positives).
BMP (Below Minimum Performance) ratings correspond to a not expected result with a false positive in healthy level and/or deviation from the expected result for the medium/high levels grouped.
The results are presented in table $n^{\circ} 7$ and distribution of rating is presented figure 6.
Table n ${ }^{\circ}$ 7: Computations of laboratories and rating


Figure 6: Distribution of rating


The distribution of rating is divided between the letter A and BMP.
The A letter represents:
48\% for Alternaria linicola
62\% for Botrytis cinerea
95\% for Colletotrichum lini
The BMP rating is due to:

- false positive in the healthy lot
- false negative in medium or high lot


### 2.2 Quantitative results

### 2.2.1 Statistical tools

Due to the high variability of the results obtained by the laboratories, the statistical analysis with the Z-score was not adapted. We chose to use the Box plot presentation to analyze the data

## - 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. Values given by participants have been compared to values obtained during homogeneity and stability tests and with a group of participants (all results).

## - Rating of laboratories

We chose to use the Box plot presentation for rating of participants with the rule:
A: box part of the results of the lab in the limits of the box plot of homogeneity and stability tests
B: box part of the results of the lab in the limits of the box plot of homogeneity and stability tests but high heterogeneity of results or low tendency to over or underestimate compared to the box plot of homogeneity and stability tests
C: tendency to over or underestimate compared to the box plot of homogeneity and stability tests BMP: strong tendency to over or underestimate compared to the box plot of homogeneity and stability tests

### 2.2.2 Statistical analysis of data

Raw data of all laboratories are given in appendix A .
The Box plot tool was used for each pathogen for each level.

### 2.2.2.1 Alternaria linicola

The mean of the 3 replicates was performed and compared to the homogeneity and stability tests results (figure 7). The Box plot results are given for medium level in figure 8 and for high level in figure 9.

Figure 7: Mean of 3 replicates for both levels


We observed a significant variability between participants results. The difference between the lower and maximum percentage obtained by the laboratories was $6.17 \%$ for the medium level and $10 \%$ for the high level.

Figure 8: Box plot analysis for medium level


The graph shows 3 groups of results:

- Group 1: 1 laboratory has result to tendency over-estimate results (Lab 12) and a high variability of results. (B)
- Group 2: 8 laboratories have results within limit of the stability and homogeneity results. (Lab 01; Lab 02; Lab 06; Lab 07; Lab 08; Lab 17; Lab 24 and Lab 25). (A)
- Group 3: 12 laboratories have results with a tendency to under-estimate. We observed inside this group 2 levels of underestimation:
- 11 laboratories have results $\leq 2 \%$ of detection (Lab 03; Lab 04; Lab 05; Lab 09; Lab 11; Lab 14, Lab 15; Lab 16; Lab 19; Lab20; Lab 22). (BMP)
- 1 laboratory between has result $4>\%>2 \%$ (Lab 13). (B)

Figure 9: Box plot analysis for high level


The graph shows 4 groups of results

- Group 1: 1 laboratory has result within limit of the stability and homogeneity results (Lab 08) (A)
- Group 2: 2 laboratories have results close to limit of the stability and homogeneity results (Lab 06, Lab 24) (B)
- Group 3: 18 laboratories have results with a tendency to under-estimate. We observed inside this group 2 levels of underestimation:
- 9 laboratories have results $\leq$ to 2.5: Lab 03; Lab 04; Lab 05; Lab 09; Lab 11; Lab 14, Lab 16; Lab 20; Lab 22. (BMP)
- 9 laboratories have results between $7>\%>2.5$ \%: Lab 01, Lab 02; Lab 07; Lab 12, Lab 13; Lab 15 Lab 17, Lab 19 and Lab 25. (C)


## Conclusion:

The results show a significant variability between laboratories, especially for the high lot.
The results from Lab 12 show significant variability between replicates for the both levels.

### 2.2.2.2 Botrytis cinerea

The mean of 3 replicates was performed and compared with the homogeneity and stability tests in figure 10. The box plot results are given in figure 11 for medium level and figure 12 for high level.
Figure 10: Mean of 3 replicates for the both levels


We observed a significant variability between participants results. The difference between the lower and maximum percentage obtained by the laboratories was $11.16 \%$ for the medium level and $16.9 \%$ for the high level.

Figure 11: Box plot analysis for medium level


The graph shows 3 groups of results

- Group 1: 1 laboratory has result with a tendency to over-estimate (Lab 09).(BMP)
- Group 2: 8 laboratories have results close to limit of the stability and homogeneity results (Lab 01, Lab 02, Lab 06, Lab 08, Lab 14, Lab 16, Lab 17and Lab 22) (A)
- Group 3: 12 laboratories have results with a tendency to under-estimate. We observed inside this group 2 levels of underestimation:
- 3 laboratories have results between $4>\%>2 \%$ : Lab 05, Lab 7 and Lab 11 (B)
- 9 laboratories have results $\leq$ to 2: Lab 03; Lab 04, Lab 12; Lab 13; Lab 15, Lab 19; Lab 20; Lab 24 and Lab 25. (BMP)

Figure 12: Box plot analysis for high level


The graph shows 3 groups of results

- Group 1: 1 laboratory has result a tendency to over-estimate: Lab 09. (C)
- Group 2: 3 laboratories have results close to limit of the stability and homogeneity results (Lab 01, Lab 06, Lab 08) (A)
- Group 3: 14 laboratories have a tendency to under-estimate. We observed inside this group 2 levels of underestimation:
- 13 laboratories have results $\leq 5 \%$ : Lab 03; Lab 04; Lab 05; Lab 07 Lab 11; Lab 12; Lab 13; Lab 15, Lab 19; Lab 20; Lab 22, Lab 24 and Lab 25. (BMP)
- 4 laboratories have results between $7.5>\%>5.5$ \%: Lab 02, Lab 14, Lab 16, Lab 17 (B)


## Conclusion:

The results show a significant variability between laboratories, which is more visible on the high lot. The results of Lab 09 show a tendency to over-estimate for the both levels.

### 2.2.2.3 Colletotrichum lini

The mean of 3 replicates was performed and compared with the homogeneity and stability tests figure 13. The box plot results are given in figure 14 for medium level and figure 15 for high level.

Figure 13: Mean of 3 replicates for the both levels


We observed a significant variability between participants results. The difference between the lower and maximum percentage obtained by the laboratories was $7.6 \%$ for the medium level and $16.5 \%$ for the high level.

Figure 14: Box plot analysis for medium level


The graph shows 3 groups of results

- Group 1: 18 laboratories have results within limit of the stability and homogeneity results: Lab 01, Lab 02, Lab 03; Lab 05, Lab 06, Lab 07, Lab 08, Lab 09, Lab 11, Lab 13, Lab 14, Lab 15, Lab 16, Lab 17, Lab 19, Lab 22, Lab 24 and Lab 25. (A)
- Group 2: 2 laboratories have results with a tendency to under-estimate: Lab 04; Lab 20 (B)
- Group 3: 1 laboratory which do not detect Lab 12. (BMP)

Lab 05, Lab 15, Lab 09 and Lab 22 had a variability between the repetition

Figure 15: Box plot analysis for high level


The graph shows 2 groups of results

- Group 1: 15 laboratories have results within limit of the stability and homogeneity results: Lab 01, Lab 02, Lab 03; Lab 06, Lab 07, Lab 08, Lab 09, Lab 11, Lab 13, Lab 14, Lab 15, Lab 16, Lab 17, Lab 22, Lab 24. (A)
- Group 2: 6 laboratories have results with a tendency to under-estimate: Lab 04, Lab 05, Lab 19, Lab 20; Lab 25 (B) and Lab 12 (BMP).


## Conclusion:

The results show a variability between laboratories, which is more visible on the high lot.

### 2.2.3 Rating: Z-score-computations and rating system

Due to the variability of the results of laboratories, this statistical tool cannot be applied.
We propose a rating based on the box plot is or/and not in the limit between mini and maxi value all of homogeneity and stability values. The rule of decision of the rating are:

- A rating = box plot between within limits
- $\quad$ B rating $=$ close to limits
- $\quad$ C rating $=$ under the limits
- BMP rating = close to $0 \%$

The value of participant corresponds of the mean of 3 samples/pathogen/level and are presented in table $\mathrm{n}^{\circ} 8$ and distribution of rating is presented figure 16.

Table $\mathrm{n}^{\circ}$ 8: Overview of ratings

| Lab number | Participation | Quantitative rating |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Alternaria linicola |  | Botrytis cinerea |  | Colletotrichum lini |  |
|  |  | Medium | High | Medium | High | Medium | High |
| 01 | obligatory | A | C | A | A | A | A |
| 02 | obligatory | A | C | A | B | A | A |
| 03 | obligatory | BMP | BMP | BMP | BMP | A | A |
| 04 | obligatory | BMP | BMP | BMP | BMP | B | B |
| 05 | obligatory | BMP | BMP | B | BMP | A | B |
| 06 | obligatory | A | B | A | A | A | A |
| 07 | obligatory | A | C | B | BMP | A | A |
| 08 | obligatory | A | A | A | A | A | A |
| 09 | obligatory | BMP | BMP | BMP | C | A | A |
| 11 | obligatory | BMP | BMP | B | BMP | A | A |
| 12 | voluntary | B | C | BMP | BMP | BMP | BMP |
| 13 | voluntary | B | C | BMP | BMP | A | A |
| 14 | voluntary | BMP | BMP | A | B | A | A |
| 15 | voluntary | BMP | C | BMP | BMP | A | A |
| 16 | voluntary | BMP | BMP | A | B | A | A |
| 17 | voluntary | A | C | A | B | A | A |
| 19 | voluntary | BMP | C | BMP | BMP | A | B |
| 20 | voluntary | BMP | BMP | A | BMP | A | B |
| 22 | voluntary | BMP | BMP | A | BMP | A | A |
| 24 | voluntary | A | B | BMP | BMP | A | A |
| 25 | voluntary | A | C | BMP | BMP | A | B |

Figure 16: Distribution of rating


Whatever the pathogen, the A rating obtained for the medium level is higher than the high level.
The percentage of laboratories obtaining an A rating was for each pathogen:

| Pathogen | Medium | High |
| :--- | :---: | :---: |
| Alternaria linicola | 38 | 5 |
| Botrytis cinerea | 43 | 14 |
| Colletotrichum lini | 90 | 71 |

Alternaria linicola and Botrytis cinerea were the less detected for both levels. The Colletotrichum lini was the pathogen better detected.

### 2.2.4 Rating final

The table is a summary of the different results in table $\mathrm{n}^{\circ} 9$ and figure 17 shows the distribution.
Table n ${ }^{\circ} 9$ : summary of the different results

| Lab number | Participation | Alternaria linicola |  |  |  | Botrytis cinerea |  |  |  | Colletotrichum lini |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Qualitative | Quantitative |  | Final | Qualitative | Quantitative |  | Final | Qualitative | Quantitative |  | Final |
|  |  |  | Medium | High |  |  | Medium | High |  |  | Medium | High |  |
| 01 | obligatory | A | A | C | C | A | A | A | A | A | A | A | A |
| 02 | obligatory | A | A | C | C | A | A | B | B | A | A | A | A |
| 03 | obligatory | BMP | BMP | BMP | BMP | A | BMP | BMP | BMP | A | A | A | A |
| 04 | obligatory | BMP | BMP | BMP | BMP | A | BMP | BMP | BMP | A | B | B | B |
| 05 | obligatory | BMP | BMP | BMP | BMP | A | B | BMP | BMP | A | A | B | B |
| 06 | obligatory | A | A | B | B | A | A | A | A | A | A | A | A |
| 07 | obligatory | A | A | C | C | A | B | BMP | BMP | A | A | A | A |
| 08 | obligatory | A | A | A | A | A | A | A | A | A | A | A | A |
| 09 | obligatory | BMP | BMP | BMP | BMP | BMP | BMP | C | BMP | A | A | A | A |
| 11 | obligatory | BMP | BMP | BMP | BMP | A | B | BMP | BMP | A | A | A | A |
| 12 | voluntary | BMP | B | C | BMP | BMP | BMP | BMP | BMP | BMP | BMP | BMP | BMP |
| 13 | voluntary | A | B | C | C | BMP | BMP | BMP | BMP | A | A | A | A |
| 14 | voluntary | BMP | BMP | BMP | BMP | A | A | B | B | A | A | A | A |
| 15 | voluntary | BMP | BMP | C | BMP | BMP | BMP | BMP | BMP | A | A | A | A |
| 16 | voluntary | BMP | BMP | BMP | BMP | A | A | B | B | A | A | A | A |
| 17 | voluntary | A | A | C | C | A | A | B | B | A | A | A | A |
| 19 | voluntary | A | BMP | C | BMP | A | BMP | BMP | BMP | A | A | B | B |
| 20 | voluntary | BMP | BMP | BMP | BMP | BMP | A | BMP | BMP | A | A | B | B |
| 22 | voluntary | BMP | BMP | BMP | BMP | BMP | A | BMP | BMP | A | A | A | A |
| 24 | voluntary | A | A | B | B | BMP | BMP | BMP | BMP | A | A | A | A |
| 25 | voluntary | A | A | C | C | BMP | BMP | BMP | BMP | A | A | B | B |

Figure 17: Distribution of final rating
In the end: the distribution of ratings for this proficiency test is as follows:


The BMP rating is due to the qualitative analysis with a false or/and negative results and with a deviation under or/and over for expected results.

The summary of qualitative and quantitative results by pathogen is given in Appendix B

## - Alternaria linicola

For the healthy samples, during the pre-tests, homogeneity and stability tests, no samples were positive. In this case, we considered positive results as false positive.

We prepared the samples by spiking with contaminated seeds, we considered negative results as false negative for medium or high levels. For high level we considered that laboratories who obtained values far from the expected values underestimated.

## - Botrytis cinerea

For the healthy samples, during pre-tests, homogeneity and stability tests, no samples are positive detected. In this case, the laboratory made false positive samples.

The contamination is artificial, we know the number of seeds added, so the expected result.
Accredited laboratories detect positive and negative samples as expected, except for the laboratory (Lab 09) which presents a false positive and a tendency to overestimate the $\%$ for both levels of contamination.

Many laboratories tend to underestimate for the both levels.

## - Colletotrichum lini

For the healthy samples, during pre-tests, homogeneity and stability tests, no samples are positive detected, and no laboratories made a false positives result.

In qualitative analysis, all accredited laboratories are conformed. One laboratory (Lab 12) didn't detect the pathogen on the both levels however we prepared the samples with two types of contamination natural infection for medium level and artificial contamination for high level of contamination.

In quantitative results: 7 accredited laboratories obtained the conform results. It demonstrates a good knowledge of this pathogen.

## 3 CONCLUSION:

Colletotrichum lini was better detected with no false positive samples. The identification criteria are typical (orange color) and cannot be confused with other pathogens or saprophytes.

Botrytis cinerea was artificially inoculated and the time between contamination and analysis, less than 3 months, does not induce a decrease of level of contamination. This was confirmed by the stability test. Some participants indicated that the fungus was not sporulated. It could be the case on media, but the sporulation is not the only criteria for identification.

Alternaria linicola is the pathogen that has been the less detected. This fungus can be confused with other species of Alternaria and overestimated or underestimated in case of no sporulation

The presence of saprophytic fungi (i.e Rhizopus) could explain the underestimation. Two laboratories (Lab 13 and Lab 15) reported problems with the temperature of the growth chamber.

3 laboratories did not follow the ISTA method. A voluntary laboratory used a blotter media and 2 laboratories made a superficial disinfection. Their results could not be related to the use of another method.


## Appendix A:

Raw data for detection laboratories

| Participation | Lab number | lot | Number of sample | \% |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Alternaria linicola |  | Botrytis cinerea |  | Colletotricum linicola |  |
|  |  |  |  | Obtained results | Mean | Obtained results | mean | Obtained results | mean |
| obligatory | Lab 01 | A_ST_1 | 73 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 213 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 264 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 17 | 4.60 | 4.20 | 4.86 | 4.45 | 4.86 | 5.87 |
|  |  | B_ST_2 | 180 | 4.00 |  | 3.50 |  | 6.00 |  |
|  |  | B_ST_3 | 220 | 4.00 |  | 5.00 |  | 6.75 |  |
|  |  | C_ST_1 | 4 | 3.28 | 3.78 | 7.58 | 9.17 | 9.34 | 12.28 |
|  |  | C_ST_2 | 142 | 3.79 |  | 9.85 |  | 15.15 |  |
|  |  | C_ST_3 | 159 | 4.28 |  | 10.08 |  | 12.34 |  |
| obligatory | Lab 02 | A_ST_1 | 56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 69 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 172 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 3 | 3.75 | 3.92 | 4.00 | 4.08 | 6.25 | 5.67 |
|  |  | B_ST_2 | 198 | 3.50 |  | 3.75 |  | 4.50 |  |
|  |  | B_ST_3 | 229 | 4.50 |  | 4.50 |  | 6.25 |  |
|  |  | C_ST_1 | 61 | 6.75 | 5.17 | 8.00 | 7.33 | 12.50 | 13.50 |
|  |  | C_ST_2 | 68 | 4.00 |  | 8.00 |  | 16.00 |  |
|  |  | C_ST_3 | 197 | 4.75 |  | 6.00 |  | 12.00 |  |
| obligatory | Lab 03 | A_ST_1 | 112 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 175 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 200 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 57 | 0.00 | 0.00 | 0.50 | 0.67 | 6.00 | 5.93 |
|  |  | B_ST_2 | 122 | 0.00 |  | 1.00 |  | 5.75 |  |
|  |  | B_ST_3 | 168 | 0.00 |  | 0.50 |  | 6.05 |  |
|  |  | C_ST_1 | 48 | 0.75 | 0.67 | 1.75 | 2.09 | 12.25 | 11.19 |
|  |  | C_ST_2 | 52 | 0.00 |  | 2.50 |  | 9.75 |  |
|  |  | C_ST_3 | 224 | 1.26 |  | 2.01 |  | 11.56 |  |
| obligatory | Lab 04 | A_ST_1 | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 10 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 21 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 171 | 0.00 | 0.25 | 1.00 | 0.92 | 3.00 | 2.42 |
|  |  | B_ST_2 | 211 | 0.50 |  | 1.00 |  | 1.25 |  |
|  |  | B_ST_3 | 226 | 0.25 |  | 0.75 |  | 3.00 |  |
|  |  | C_ST_1 | 22 | 2.00 | 1.58 | 3.00 | 2.25 | 5.50 | 5.75 |
|  |  | C_ST_2 | 162 | 2.25 |  | 2.75 |  | 6.00 |  |
|  |  | C_ST_3 | 214 | 0.50 |  | 1.00 |  | 5.75 |  |
| obligatory | Lab 05 | A_ST_1 | 25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 207 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 217 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 157 | 0.00 | 0.00 | 3.25 | 2.08 | 3.50 | 5.58 |
|  |  | B_ST_2 | 234 | 0.00 |  | 1.25 |  | 6.50 |  |
|  |  | B_ST_3 | 243 | 0.00 |  | 1.75 |  | 6.75 |  |
|  |  | C_ST_1 | 51 | 0.00 | 0.00 | 6.50 | 4.50 | 8.75 | 7.92 |
|  |  | C_ST_2 | 190 | 0.00 |  | 3.50 |  | 7.00 |  |
|  |  | C_ST_3 | 261 | 0.00 |  | 3.50 |  | 8.00 |  |
| obligatory | Lab 06 | A_ST_1 | 37 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 114 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 173 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 66 | 5.00 | 5.33 | 3.75 | 3.75 | 7.50 | 6.42 |
|  |  | B_ST_2 | 141 | 5.00 |  | 4.25 |  | 6.75 |  |
|  |  | B_ST_3 | 166 | 6.00 |  | 3.25 |  | 5.00 |  |
|  |  | C_ST_1 | 16 | 8.50 | 8.42 | 8.00 | 9.17 | 13.00 | 13.58 |
|  |  | C_ST_2 | 93 | 8.50 |  | 8.75 |  | 15.75 |  |
|  |  | C_ST_3 | 181 | 8.25 |  | 10.75 |  | 12.00 |  |
| obligatory | Lab 07 | A_ST_1 | 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 236 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 257 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 59 | 5.00 | 4.83 | 2.40 | 1.92 | 7.10 | 6.98 |
|  |  | B_ST_2 | 70 | 4.50 |  | 1.60 |  | 7.60 |  |
|  |  | B_ST_3 | 242 | 5.00 |  | 1.75 |  | 6.25 |  |
|  |  | C_ST_1 | 41 | 6.00 | 6.30 | 4.90 | 4.37 | 11.70 | 13.73 |
|  |  | C_ST_2 | 78 | 7.10 |  | 3.20 |  | 15.80 |  |
|  |  | C_ST_3 | 132 | 5.80 |  | 5.00 |  | 13.70 |  |


| Participation | Lab number | lot | Number of sample | \% |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Alternaria linicola |  | Botrytis cinerea |  | Colletotricum linicola |  |
|  |  |  |  | Obtained results | Mean | Obtained results | mean | Obtained results | mean |
| obligatory | Lab 08 | A_ST_1 | 67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 88 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 184 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 58 | 4.25 | 4.92 | 4.75 | 4.00 | 5.75 | 5.83 |
|  |  | B_ST_2 | 108 | 6.25 |  | 4.75 |  | 5.75 |  |
|  |  | B_ST_3 | 251 | 4.25 |  | 2.50 |  | 6.00 |  |
|  |  | C_ST_1 | 36 | 10.00 | 10.25 | 9.25 | 8.50 | 13.50 | 14.67 |
|  |  | C_ST_2 | 53 | 9.75 |  | 7.25 |  | 14.00 |  |
|  |  | C_ST_3 | 259 | 11.00 |  | 9.00 |  | 16.50 |  |
| obligatory | Lab 09 | A_ST_1 | 35 | 0.00 | 0.58 | 0.00 | 0.83 | 0.00 | 0.00 |
|  |  | A_ST_2 | 54 | 1.25 |  | 1.25 |  | 0.00 |  |
|  |  | A_ST_3 | 265 | 0.50 |  | 1.25 |  | 0.00 |  |
|  |  | B_ST_1 | 33 | 0.50 | 0.67 | 9.50 | 11.58 | 5.75 | 4.92 |
|  |  | B_ST_2 | 72 | 0.00 |  | 14.00 |  | 2.75 |  |
|  |  | B_ST_3 | 119 | 1.50 |  | 11.25 |  | 6.25 |  |
|  |  | C_ST_1 | 38 | 1.00 | 0.58 | 12.25 | 17.00 | 10.75 | 10.50 |
|  |  | C_ST_2 | 50 | 0.00 |  | 20.00 |  | 6.50 |  |
|  |  | C_ST_3 | 228 | 0.75 |  | 18.75 |  | 14.25 |  |
| obligatory | Lab 11 | A_ST_1 | 71 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 169 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 267 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 145 | 1.25 | 1.42 | 2.50 | 2.75 | 6.50 | 5.42 |
|  |  | B_ST_2 | 163 | 1.75 |  | 3.25 |  | 4.00 |  |
|  |  | B_ST_3 | 232 | 1.25 |  | 2.50 |  | 5.75 |  |
|  |  | C_ST_1 | 46 | 0.00 | 0.42 | 4.50 | 3.67 | 12.50 | 12.17 |
|  |  | C_ST_2 | 64 | 0.75 |  | 3.75 |  | 13.00 |  |
|  |  | C_ST_3 | 135 | 0.50 |  | 2.75 |  | 11.00 |  |
| voluntary | Lab 12 | A_ST_1 | 104 | 0.50 | 0.67 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 156 | 1.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 158 | 0.50 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 43 | 5.00 | 6.42 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | B_ST_2 | 221 | 3.50 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_3 | 269 | 10.75 |  | 0.00 |  | 0.00 |  |
|  |  | C_ST_1 | 77 | 10.50 | 6.50 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | C_ST_2 | 89 | 3.00 |  | 0.00 |  | 0.00 |  |
|  |  | C_ST_3 | 165 | 6.00 |  | 0.00 |  | 0.00 |  |
| voluntary | Lab 13 | A_ST_1 | 91 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_ 2 | 201 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_ 3 | 250 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 155 | 2.00 | 3.10 | 1.00 | 0.59 | 6.50 | 6.19 |
|  |  | B_ST_2 | 196 | 4.51 |  | 0.00 |  | 5.51 |  |
|  |  | B_ST_3 | 225 | 2.78 |  | 0.76 |  | 6.57 |  |
|  |  | C_ST_1 | 63 | 5.38 | 4.58 | 1.03 | 0.86 | 9.23 | 9.94 |
|  |  | C_ST_2 | 65 | 4.10 |  | 1.54 |  | 12.31 |  |
|  |  | C_ST_3 | 218 | 4.27 |  | 0.00 |  | 8.29 |  |
| voluntary | Lab 14 | A_ST_1 | 27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_ 2 | 189 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 216 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 1 | 0.50 | 1.08 | 5.25 | 4.25 | 4.25 | 4.83 |
|  |  | B_ST_2 | 116 | 1.50 |  | 2.00 |  | 5.50 |  |
|  |  | B_ST_3 | 222 | 1.25 |  | 5.50 |  | 4.75 |  |
|  |  | C_ST_1 | 13 | 0.00 | 0.00 | 12.00 | 6.52 | 14.75 | 12.21 |
|  |  | C_ST_2 | 176 | 0.00 |  | 3.55 |  | 9.64 |  |
|  |  | C_ST_3 | 102 | 0.00 |  | 4.00 |  | 12.25 |  |
| voluntary | Lab 15 | A_ST_1 | 7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_ 2 | 101 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 254 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 188 | 1.75 | 1.00 | 0.00 | 0.00 | 9.00 | 6.25 |
|  |  | B_ST_2 | 203 | 1.25 |  | 0.00 |  | 6.25 |  |
|  |  | B_ST_3 | 252 | 0.00 |  | 0.00 |  | 3.50 |  |
|  |  | C_ST_1 | 195 | 3.25 | 3.25 | 0.00 | 0.00 | 10.75 | 9.33 |
|  |  | C_ST_2 | 227 | 4.75 |  | 0.00 |  | 10.50 |  |
|  |  | C_ST_3 | 239 | 1.75 |  | 0.00 |  | 6.75 |  |
| voluntary | Lab 16 | A_ST_1 | 81 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_ 2 | 138 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 270 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 80 | 1.27 | 1.26 | 4.30 | 4.53 | 5.57 | 5.29 |
|  |  | B_ST_2 | 179 | 1.75 |  | 4.75 |  | 6.00 |  |
|  |  | B_ST_3 | 248 | 0.76 |  | 4.55 |  | 4.29 |  |
|  |  | C_ST_1 | 45 | 0.00 | 0.25 | 7.14 | 7.04 | 10.71 | 9.92 |
|  |  | C_ST_2 | 210 | 0.00 |  | 7.69 |  | 10.51 |  |
|  |  | C_ST_3 | 268 | 0.75 |  | 6.28 |  | 8.54 |  |
| voluntary | Lab 17 | A_ST_1 | 177 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_ 2 | 191 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 245 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 49 | 5.51 | 6.09 | 4.51 | 3.34 | 6.27 | 5.92 |
|  |  | B_ST_2 | 107 | 7.00 |  | 2.00 |  | 6.75 |  |
|  |  | B_ST_3 | 246 | 5.75 |  | 3.50 |  | 4.75 |  |
|  |  | C_ST_1 | 9 | 3.75 | 4.92 | 5.00 | 5.92 | 10.75 | 10.67 |
|  |  | C_ST_2 | 143 | 5.00 |  | 5.00 |  | 11.50 |  |
|  |  | C_ST_3 | 255 | 6.00 |  | 7.75 |  | 9.75 |  |


| Participation | Lab number | lot | Number of sample | Alternaria linicola |  | Botrytis cinerea |  | Colletotricum linicola |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Obtained results | Mean | Obtained results | mean | Obtained results | mean |
| voluntary | Lab 19 | A_ST_1 | 11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 75 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 136 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 40 | 1.00 | 1.58 | 0.50 | 0.42 | 4.50 | 4.50 |
|  |  | B_ST_2 | 55 | 1.75 |  | 0.50 |  | 3.50 |  |
|  |  | B_ST_3 | 235 | 2.00 |  | 0.25 |  | 5.50 |  |
|  |  | C_ST_1 | 29 | 2.25 | 2.58 | 1.00 | 0.83 | 7.75 | 6.67 |
|  |  | C_ST_2 | 110 | 3.00 |  | 0.25 |  | 5.25 |  |
|  |  | C_ST_3 | 231 | 2.50 |  | 1.25 |  | 7.00 |  |
| voluntary | Lab 20 | A_ST_1 | 20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 30 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 92 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 192 | 0.75 | 0.92 | 1.25 | 1.17 | 2.75 | 2.42 |
|  |  | B_ST_2 | 215 | 0.75 |  | 1.00 |  | 2.25 |  |
|  |  | B_ST_3 | 262 | 1.25 |  | 1.25 |  | 2.25 |  |
|  |  | C_ST_1 | 82 | 0.00 | 0.58 | 0.00 | 0.08 | 5.75 | 4.33 |
|  |  | C_ST_2 | 96 | 0.75 |  | 0.00 |  | 2.50 |  |
|  |  | C_ST_3 | 139 | 1.00 |  | 0.25 |  | 4.75 |  |
| voluntary | Lab 22 | A_ST_1 | 26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 62 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 205 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 24 | 2.00 | 1.50 | 5.50 | 6.00 | 1.50 | 4.00 |
|  |  | B_ST_2 | 127 | 0.00 |  | 4.00 |  | 4.00 |  |
|  |  | B_ST_3 | 185 | 2.50 |  | 8.50 |  | 6.50 |  |
|  |  | C_ST_1 | 170 | 1.50 | 1.00 | 0.00 | 1.33 | 11.00 | 13.33 |
|  |  | C_ST_2 | 219 | 1.50 |  | 3.00 |  | 16.00 |  |
|  |  | C_ST_3 | 253 | 0.00 |  | 1.00 |  | 13.00 |  |
| voluntary | Lab 24 | A_ST_1 | 94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 123 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 183 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 32 | 4.25 | 3.92 | 1.00 | 0.42 | 7.00 | 6.00 |
|  |  | B_ST_2 | 149 | 4.50 |  | 0.00 |  | 6.25 |  |
|  |  | B_ST_3 | 199 | 3.00 |  | 0.25 |  | 4.75 |  |
|  |  | C_ST_1 | 106 | 7.25 | 7.25 | 0.00 | 1.83 | 14.25 | 13.67 |
|  |  | C_ST_2 | 131 | 8.00 |  | 3.50 |  | 12.25 |  |
|  |  | C_ST_3 | 152 | 6.50 |  | 2.00 |  | 14.50 |  |
| voluntary | Lab 25 | A_ST_1 | 147 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A_ST_2 | 212 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A_ST_3 | 237 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | B_ST_1 | 109 | 4.00 | 4.67 | 1.00 | 0.67 | 4.00 | 4.00 |
|  |  | B_ST_2 | 115 | 5.00 |  | 1.00 |  | 5.00 |  |
|  |  | B_ST_3 | 167 | 5.00 |  | 0.00 |  | 3.00 |  |
|  |  | C_ST_1 | 83 | 6.00 | 3.67 | 2.00 | 1.00 | 3.00 | 5.00 |
|  |  | C_ST_2 | 113 | 3.00 |  | 0.00 |  | 8.00 |  |
|  |  | C_ST_3 | 129 | 2.00 |  | 1.00 |  | 4.00 |  |
|  |  | A homog_ST_1 | 280 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A homog_ST_2 | 282 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_3 | 288 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_4 | 294 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_5 | 296 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_6 | 297 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_7 | 302 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_8 | 311 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_9 | 315 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_10 | 317 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A homog_ST_1 | 272 | 4.00 | 4.91 | 4.75 | 5.28 | 6.00 | 5.91 |
|  |  | A homog_ST_2 | 274 | 3.75 |  | 6.00 |  | 6.00 |  |
|  |  | A homog_ST_3 | 290 | 3.25 |  | 4.25 |  | 7.00 |  |
|  |  | A homog_ST_4 | 303 | 5.00 |  | 6.00 |  | 8.50 |  |
|  |  | A homog_ST_5 | 306 | 5.00 |  | 6.00 |  | 4.25 |  |
|  |  | A homog_ST_6 | 307 | 6.50 |  | 5.50 |  | 7.00 |  |
|  |  | A homog_ST_7 | 310 | 5.00 |  | 3.75 |  | 4.00 |  |
|  |  | A homog_ST_8 | 321 | 5.00 |  | 5.25 |  | 5.00 |  |
|  |  | A homog_ST_9 | 323 | 5.84 |  | 5.84 |  | 5.08 |  |
|  |  | A homog_ST_10 | 329 | 5.75 |  | 5.50 |  | 6.25 |  |
|  |  | A homog_ST_1 | 273 | 9.00 | 10.28 | 10.25 | 10.75 | 13.50 | 13.88 |
|  |  | A homog_ST_2 | 281 | 10.25 |  | 9.00 |  | 13.75 |  |
|  |  | A homog_ST_3 | 284 | 10.00 |  | 10.00 |  | 14.75 |  |
|  |  | A homog_ST_4 | 285 | 10.75 |  | 12.00 |  | 15.75 |  |
|  |  | A homog_ST_5 | 286 | 10.25 |  | 11.25 |  | 16.00 |  |
|  |  | A homog_ST_6 | 287 | 9.50 |  | 11.75 |  | 12.75 |  |
|  |  | A homog_ST_7 | 292 | 11.75 |  | 13.00 |  | 10.50 |  |
|  |  | A homog_ST_8 | 308 | 11.50 |  | 9.50 |  | 13.00 |  |
|  |  | A homog_ST_9 | 309 | 10.50 |  | 11.00 |  | 14.75 |  |
|  |  | A homog_ST_10 | 320 | 9.25 |  | 9.75 |  | 14.00 |  |
|  | Stability | A stab_ST_1 | 276 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  | A stab_ST_ 2 | 291 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A stab_ST_3 | 295 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A stab_ST 4 | 324 | 0.00 |  | 0.00 |  | 0.00 |  |
|  |  | A stab_ST_5 | 327 | 0.00 |  | 0.00 |  | 0.00 |  |
|  | Stability | A stab_ST_1 | 279 | 5.76 | 5.26 | 5.01 | 4.96 | 5.76 | 5.76 |
|  |  | A stab_ST_ 2 | 298 | 5.28 |  | 5.78 |  | 6.03 |  |
|  |  | A stab_ST_ 3 | 300 | 5.50 |  | 3.50 |  | 4.75 |  |
|  |  | A stab_ST_ 4 | 305 | 5.00 |  | 5.25 |  | 5.50 |  |
|  |  | A stab_ST_5 | 314 | 4.75 |  | 5.25 |  | 6.75 |  |
|  | Stability | A stab_ST_1 | 278 | 10.25 | 10.00 | 9.50 | 11.15 | 12.75 | 14.20 |
|  |  | A stab_ST_ 2 | 283 | 8.75 |  | 11.75 |  | 15.50 |  |
|  |  | A stab_ST_3 | 316 | 10.50 |  | 12.00 |  | 15.50 |  |
|  |  | A stab_ST_4 | 322 | 10.00 |  | 10.75 |  | 15.25 |  |

## Appendix B:

Summary of qualitative and quantitative results (number in bold indicates accredited laboratories)

| Lab number | Alternaria linicola |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Qualitative |  |  | Quantitatif |  |
|  | false positive | false negative |  |  |  |
|  |  | Medium | High | Medium | High |
| 01 | in line | in line | in line | central | underestimate |
| 02 | in line | in line | in line | central | underestimate |
| 03 | in line | $0^{+} / 3$ | 2+/3 | underestimate | underestimate |
| 04 | in line | $1{ }^{+} / 3$ | in line | underestimate | underestimate |
| 05 | in line | $0^{+} / 3$ | $0^{+} / 3$ | underestimate | underestimate |
| 06 | in line | in line | in line | central | underestimate |
| 07 | in line | in line | in line | central | underestimate |
| 08 | in line | in line | in line | central | central |
| 09 | $2^{+} / 0$ | $1^{+} / 3$ | $2^{+} / 3$ | underestimate | underestimate |
| 11 | in line | in line | $2^{+} / 3$ | underestimate | underestimate |
| 12 | $3^{+} / 0$ | in line | in line | overestimate | underestimate |
| 13 | in line | in line | in line | underestimate | underestimate |
| 14 | in line | in line | 0+/3 | underestimate | underestimate |
| 15 | in line | $1^{+} / 3$ | in line | underestimate | underestimate |
| 16 | in line | in line | $1^{+} / 3$ | underestimate | underestimate |
| 17 | in line | in line | in line | central | underestimate |
| 19 | in line | in line | in line | underestimate | underestimate |
| 20 | in line | in line | $2^{+} / 3$ | underestimate | underestimate |
| 22 | in line | $1^{+} / 3$ | $2^{+} / 3$ | underestimate | underestimate |
| 24 | in line | in line | in line | central | underestimate |
| 25 | in line | in line | in line | central | underestimate |


| Lab number | Botrytis cinerea |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Qualitative |  |  | Quantitatif |  |
|  | false positive | false negative |  |  |  |
|  |  | Medium | High | Medium | High |
| 01 | in line | in line | in line | central | central |
| 02 | in line | in line | in line | central | underestimate |
| 03 | in line | in line | in line | underestimate | underestimate |
| 04 | in line | in line | in line | underestimate | underestimate |
| 05 | in line | in line | in line | underestimate | underestimate |
| 06 | in line | in line | in line | central | central |
| 07 | in line | in line | in line | underestimate | underestimate |
| 08 | in line | in line | in line | central | underestimate |
| 09 | $2^{+} / 3$ | in line | in line | overestimate | overestimate |
| 11 | in line | in line | in line | underestimate | underestimate |
| 12 | in line | $0^{+} / 3$ | $0^{+} / 3$ | underestimate | underestimate |
| 13 | in line | $2^{+} / 3$ | $2^{+} / 3$ | underestimate | underestimate |
| 14 | in line | in line | in line | central | underestimate |
| 15 | in line | $0^{+} / 3$ | $0^{+} / 3$ | underestimate | underestimate |
| 16 | in line | in line | in line | central | underestimate |
| 17 | in line | in line | in line | central | underestimate |
| 19 | in line | in line | in line | underestimate | underestimate |
| 20 | in line | in line | $1^{+} / 3$ | underestimate | underestimate |
| 22 | in line | in line | $2^{+} / 3$ | central | underestimate |
| 24 | in line | $2^{+} / 3$ | $2^{+} / 3$ | underestimate | underestimate |
| 25 | in line | $2^{+} / 3$ | $2^{+} / 3$ | underestimate | underestimate |


| Lab number | Colletotrichum lini |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Qualitative |  |  | Quantitatif |  |
|  | false positive | false negative Medium | false negative High |  |  |
|  |  |  |  | Medium | High |
| 01 | in line | in line | in line | central | central |
| 02 | in line | in line | in line | central | central |
| 03 | in line | in line | in line | central | central |
| 04 | in line | in line | in line | underestimate | underestimate |
| 05 | in line | in line | in line | central | underestimate |
| 06 | in line | in line | in line | central | central |
| 07 | in line | in line | in line | central | central |
| 08 | in line | in line | in line | central | central |
| 09 | in line | in line | in line | central | central |
| 11 | in line | in line | in line | central | central |
| 12 | in line | 0+/3 | 0+/3 | underestimate | underestimate |
| 13 | in line | in line | in line | central | central |
| 14 | in line | in line | in line | central | central |
| 15 | in line | in line | in line | central | central |
| 16 | in line | in line | in line | central | central |
| 17 | in line | in line | in line | central | central |
| 19 | in line | in line | in line | central | underestimate |
| 20 | in line | in line | in line | underestimate | underestimate |
| 22 | in line | in line | in line | central | central |
| 24 | in line | in line | in line | central | central |
| 25 | in line | in line | in line | central | underestimate |

