



## **Performance Data Evaluation for Specified Trait Purity**

Version 1.1

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## Performance Data Evaluation for Specified Trait Purity

### 1 SUMMARY

This document describes how to provide information to ISTA auditors when a laboratory claims for accreditation on the ability for determining a specified trait by the mean of seed testing, when the performance based approach has been chosen by the laboratory.

This is related to Chapter 8 of the ISTA Rules, for testing purity of seed lots with specified traits, as no standardised method is in the rules for this type of test.

The laboratory shall specify the specified trait, and give information on the validated method that it will use to test seeds.

The samples used must be seeds.

The laboratory shall show evidence on the ability to detect the specified trait, and to quantify the specified trait in a range from 80% to 100%.

### 2 GENERAL INFORMATION ON SPECIFIED TRAIT(S) AND METHOD

The specified trait(s) shall be defined by the laboratory (e.g., Cry3Bb1 protein).

Specified trait(s) as described by the laboratory	Comments

The method used shall be described by the laboratory to a certain extent, to allow auditors to understand how results are obtained.

The method is chosen by the laboratory (e.g., lateral flow strip, herbicide towel test, seedling spray test, insect bio-assay, PCR, etc).

Description of the type of method used by the laboratory	Comments

This document applies when a laboratory thinks it is ready to manage properly routine testing and it wishes to show evidence of this.

Thus, the way to validate the method, and to install it properly in the laboratory is not to be described in this document. The laboratory shall only give a short summary, and give appropriate references.

Short statement made by the lab about the way the method was validated and installed	Comments

Reference on documentation available, or other parties to contact, to be able to get information on validation of the method and its fitness for the purpose	Comments

### 3 SEEDS TO PREPARE THE SAMPLES

The samples used must be seeds.

In case the seeds are ground, the ability to obtain homogeneous and fine flour from seeds is one of the key elements to obtain a result which is representative from the sample.

In case the seeds are germinated, the percentage of germination shall be taken into account to obtain enough plants.

Samples are prepared from 2 sources of seeds which shall be in principle 100% seeds with absence of specified trait(s) on one hand, and 100% seeds with the specified trait(s) on the other hand.

ISTA recommends a 400 seeds check for the source of seed with specified trait(s) and the check of 400 seeds for the source of seed with absence of the specified trait(s).

In that case the lower bound of purity of the seeds is 99.25% with 95% confidence.

The fact that the 2 sources are pure enough shall be assessed by the laboratory. This can be done by a check as recommended above by ISTA, or by other means at the initiative of the laboratory.

Statement of the laboratory on the assessment of purity of seed source with presence of specified trait(s)	Comments

Statement of the laboratory on the assessment of purity of seed source with absence of specified trait(s)	Comments

*Approximate minimum number of seeds to gather to obtain enough seeds to prepare the samples:*

	Seeds with specified trait(s)	Seeds without specified trait(s)	step	comments
Ability to check for quantification of purity of specified trait(s)	~800	100	ability to quantify the specified trait(s) purity	9 samples of 90 seeds

### 4 QUANTIFICATION OF SPECIFIED TRAIT(S) PURITY:

Data may be provided in % seeds by number of seeds, in % seeds by mass of seeds, or in another unit, such as % DNA copies.

The performance data evaluation aims at checking purity % results in the range from 80% to 100%.

Samples are all made up of 90 seeds in total by counting out individual seeds. Samples shall not be obtained by using seed weighing methods.

A number of seeds with the specified trait(s) are spiked in the samples.

For each of 3 levels, 3 samples of 90 seeds shall be prepared, 9 samples in total.

3 levels of presence shall be selected by the person who prepares the sample, blind to the testers.

- Blind level 1 shall be in the purity interval [80%, 90%] in number of seeds (72 to 81 positive seeds spiked)
- Blind level 2 shall be in the purity interval ]90%, 95%] in number of seeds (82 to 85 positive seeds spiked)
- Blind level 3 shall be in the purity interval ]95%, 100%] in number of seeds (87 to 90 positive seeds spiked)

For each of the blind levels, the person who prepares the samples shall select at random a number of seeds within the range, and prepare 3 samples with this same number of seeds having the specified trait(s).

*The number of seeds required for the 9 samples is about:*

	Seeds with <b>specified</b> trait(s)	Seeds without <b>specified</b> trait(s)
Blind level 1	81*3= 243 max	19*3=57 max
Blind level 2	85*3= 255 max	8*3=24 max
Blind level 3	90*3= 270 max	3*3=9 max
Approx. number seeds	780	100

The 9 samples for the ability to quantify the purity of the specified trait(s) shall be randomly coded from 1 to 9 and given blind of level to the staff that will perform the tests.

Statement of the laboratory about blindness of the 9 samples
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**5 DESCRIPTION OF SAMPLES, AND REPORT OF RESULTS**

		blind level 1	blind level 2	blind level 3
Spiking level (% number of seeds)				
number of spiked seeds	sample 1			
number of spiked seeds	sample 2			
number of spiked seeds	sample 3			
number of spiked seeds	sample 4			
weight of spiked seeds	sample 1			
weight of spiked seeds	sample 2			
weight of spiked seeds	sample 3			

weight of spiked seeds	sample 4			
weight of seeds without trait(s)	sample 1			
weight of seeds without trait(s)	sample 2			
weight of seeds without trait(s)	sample 3			
weight of seeds without trait(s)	sample 4			
% other unit	sample 1			
% other unit	sample 2			
% other unit	sample 3			
% other unit	sample 4			

**Results**

result in % number of seeds	sample 1			
result in % number of seeds	sample 2			
result in % number of seeds	sample 3			
result in % number of seeds	sample 4			
result in % weight of seeds	sample 1			
result in % weight of seeds	sample 2			
result in % weight of seeds	sample 3			
result in % weight of seeds	sample 4			
result in % other unit*	sample 1			
result in % other unit*	sample 2			
result in % other unit*	sample 3			
result in % other unit*	sample 4			

\* The unit must correspond to the % other unit chosen above.

It is not permitted to give a table of results with missing samples. In case of problems with a sample, another sample of the same level shall be prepared and analysed.

The values given by the staff that performs the analysis shall be reported without any change by the person who prepared the coded samples (no retest, no re-evaluation if a value is far from the true value, etc).

Statement of the laboratory on the fact that the results obtained have been reported with no changes

Statement of the laboratory about how to derive a unit from another, if applicable

## 6 STATISTICAL EVALUATION OF PERFORMANCE DATA

Accuracy and repeatability shall be determined as an objective criteria to evaluate the performance data (for details please refer to the ISTA Handbook on Statistics in Seed Testing)

Accuracy is a way to measure closeness of agreement between a test result and the true value.

Repeatability is a measure of dispersion of test results under repeatability conditions. It will be computed as the square-root of the average of the variances of the 3 samples per level and will be expressed in % of the mean of the true levels in the test.

The three samples per level have exactly the same true value expressed as % in number of seeds if the number of seeds is counted.

*Example for computation of repeatability std-dev in % of the mean:*

True level	Sample	Result	Variance
Blind-82.2%	sample 1	80.00%	0.00053498
	sample 2	83.33%	
	sample 3	84.44%	
Blind-94.4%	sample 1	94.44%	0.00049383
	sample 2	96.67%	
	sample 3	92.22%	
Blind-97.8%	sample 1	100.00%	0.00028807
	sample 2	96.67%	
	sample 3	98.89%	
<b>Average variance (repeatability variance)</b>			0.00043896
<b>Mean of the true levels</b>			0.9146
<b>Repeatability std-dev in % of the mean</b>			<b>2.29%</b>

## 7 EVALUATION OF THE DATA SUBMITTED BY THE LABORATORY

The performance data are a part of the different elements of assessment for accreditation.

The grades given below to evaluate the performance data are a help for the auditors.

Accuracy of specified trait(s) quantification:

Accuracy is calculated as

$[(\text{observed value}) - (\text{true value})] / \text{true value}$ .

Grade 1: all 9 samples have the accuracy within +/- 2% of true value (inclusive of boundaries)

Grade 2: all 9 samples have the accuracy within +/- 4% of true value (inclusive of boundaries)

Grade 3: at least one sample has the accuracy outside +/- 4% of true value.

Repeatability of specified trait(s) quantification:

Grade 1: Repeatability std-dev in % of the mean is 1% or below

Grade 2: Repeatability std-dev in % of the mean is 3% or below

Grade 3: Repeatability std-dev in % of the mean is above 3%