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Seed Quality Assurance

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Inter laboratory comparison (ILC) report*

ISTA PT.22-SH 7-022

ISTA Proficiency test: Detection *of Microdochium nivale* and *M. majus* on *Triticum* spp.

Original report signed and archived

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1 PROFICIENCY TEST ORGANISATION

The aim of this Proficiency Test was to verify the ability of laboratories to detect and identify the pathogen *Microdochium nivale* and *M. majus* in *Triticum aestivum* (Wheat) seeds.

Schedule

Dispatch of Samples	June 2022
Deadline for submission of results	30 September 2022
Sending of report and individual letters	January 2023

Nine laboratories participated to this test and were randomly allocated a number, so that

results remained anonymous.

Of the nine participants registered for the proficiency test:

- 5 were accredited for 7-022 method.
- 4 were not accredited for 7-022 method.

1.1 Notation of results

The laboratories indicated a quantitative result on four individual replicates of colonies of target pathogen observed giving a mean quantitative result for each sample as a % of *Microdochium nivale* and/or *M. majus* observed.

1.2 Composition of the sample panel

Three samples of 400 seeds were sent to the participants at the following infection levels (mean result of homogeneity tests), shown in Table 1.

Table 1: Infection level of samples

Lot	Level of contamination	Expected Result
1	High	34%
2	Healthy	Nil or 0.25%
3	Medium	12%

1.3 Pretest

The ideal infection levels would be Nil for the healthy, 10% for the medium and 30% for the high level. Three seed lots were identified. The healthy seed lot was obtained from GEVES, and the medium and high infections were from the Scottish 2021 harvest and were tested in 400 seed samples by ISTA method 7-022. The results of pre-tests are indicated in Table 2. Samples were also checked for the presence of other pathogens to ensure that the target pathogen would not be overwhelmed. Levels of *Parastagonospora nodorum* and *Fusarium* species were very low on all three seed lots. Thousand seed weights were obtained according to current ISTA rules Chapter 10, to ensure sufficient seed was obtained for the proficiency test requirements.

Table 2: Sample infection results from pre-test

Lot	OSTS reference	% *Microdochium* infection	% *Parastagonospora nodorum*	% *Fusarium* species	Thousand seed weight (g)
3	77645	12	Nil	2	53.9
2	78871	Nil	Nil	1	53.5
1	77644	34	Nil	1	47.3

1.4 Homogeneity Test

The statistical analysis for homogeneity was carried out using Hampels Outliers test tool, to look for outlier results from the normal distribution of results of *Microdochium nivale* and *M. majus*. This Homogeneity test was carried out after packaging and just before sample dispatch. Ten extra samples of 400 seeds representing each contamination level were tested. The samples were tested on the 26th & 27th of April 2022. The raw data are given in Appendix A, part 1.

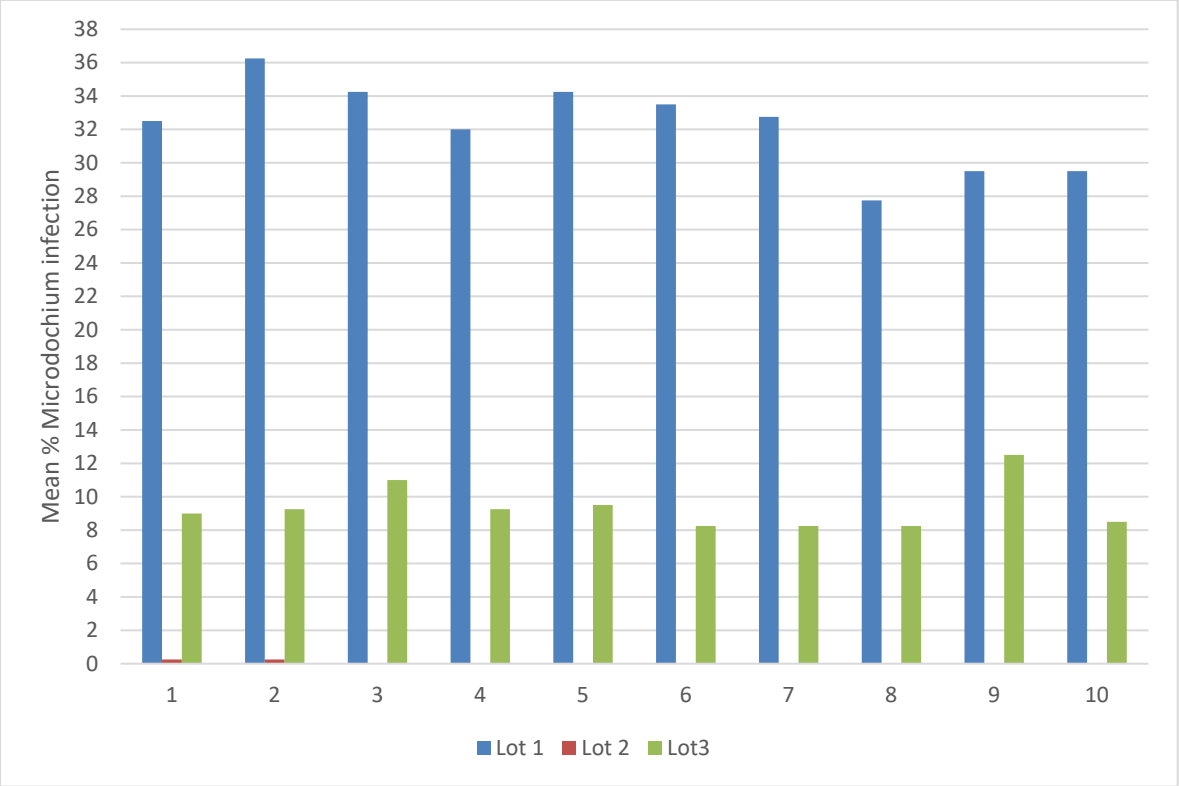


Figure 1: Mean % *Microdochium* infection levels in homogeneity test sub-samples

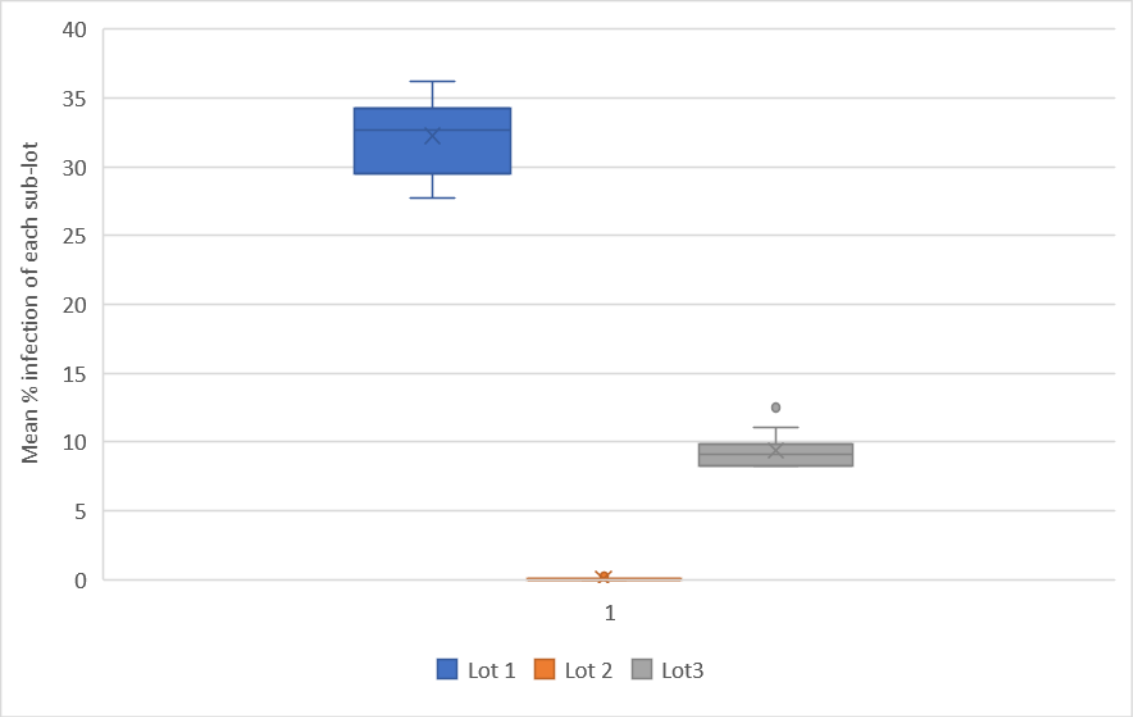


Figure 2: Box and whisker plot for the homogeneity sub-sample mean results for Lots 1, 2 & 3

1.4.1 Healthy seed lot

Two outliers were detected in sub-samples 1 and 2, but only one isolate of *Microdochium* was the cause of this. One colony could easily occur by chance, so although Hampels Outlier Tool identified this as an outlier, one colony detected is acceptable.

MS Excel Hampels Outlier Test Lot 2			Healthy			
Lab	Lab Values (Xi)	| Xi - M|	Status			
1	0.25	0.250	Outlier		**Median (M):**	**0.000**
2	0.25	0.250	Outlier		**MAD:**	**0.000**
3	0.00	0.000	OK		**5.2 X MAD**	**0.000**
4	0.00	0.000	OK			
5	0.00	0.000	OK			
6	0.00	0.000	OK			
7	0.00	0.000	OK			
8	0.00	0.000	OK			
9	0.00	0.000	OK			
10	0.00	0.000	OK			

1.4.2 Medium infection seed lot

For the medium infection level, sub-sample 9 appeared to give a high result but this was not picked out as an outlier using Hamples outlier tool.

MS Excel Hampels Outlier Test Lot 3			Medium			
Lab	Lab Values (Xi)	| Xi - M|	Status			
1	9.00	0.125	OK		**Median (M):**	**9.125**
2	9.25	0.125	OK		**MAD:**	**0.750**
3	11.00	1.875	OK		**5.2 X MAD**	**3.900**
4	9.25	0.125	OK			
5	9.50	0.375	OK			
6	8.25	0.875	OK			
7	8.25	0.875	OK			
8	8.25	0.875	OK			
9	12.50	3.375	OK			
10	8.50	0.625	OK			

1.4.3 High infection seed lot

For the high infection lot, sub-sample 8 gave the lowest result but again it was not picked out as an outlier using Hamples outlier tool.

MS Excel Hampels Outlier Test Lot 1			High			
Lab	Lab Values (Xi)	| Xi - M|	Status			
1	32.50	0.125	OK		**Median (M):**	**32.625**
2	36.25	3.625	OK		**MAD:**	**1.625**
3	34.25	1.625	OK		**5.2 X MAD**	**8.450**
4	32.00	0.625	OK			
5	34.25	1.625	OK			
6	33.50	0.875	OK			
7	32.75	0.125	OK			
8	27.75	4.875	OK			
9	29.50	3.125	OK			
10	29.50	3.125	OK			

1.5 Stability Test

The statistical analysis for the stability of pathogen infection was carried out using Hampels Outlier test tool, to look for outlier results from the normal distribution of results of *Microdochium nivale* and *M. majus*. This stability test was carried out after all participating labs had submitted their results. Three extra samples of 400 seeds representing each contamination level were tested. The samples were tested in September 2022. The raw data is given in Appendix A, part 3.

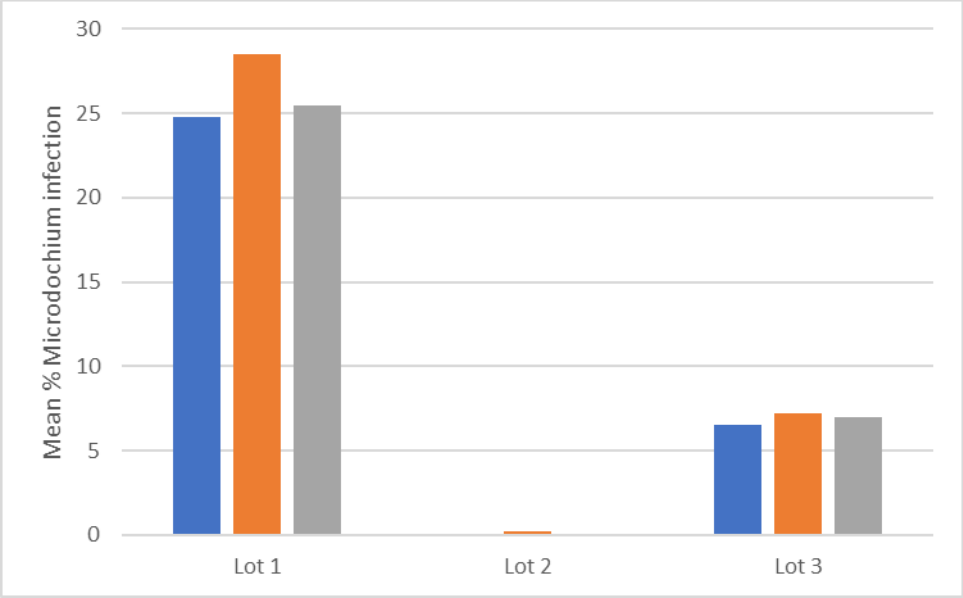


Figure 3: Mean % *Microdochium* infection levels in stability test sub-samples

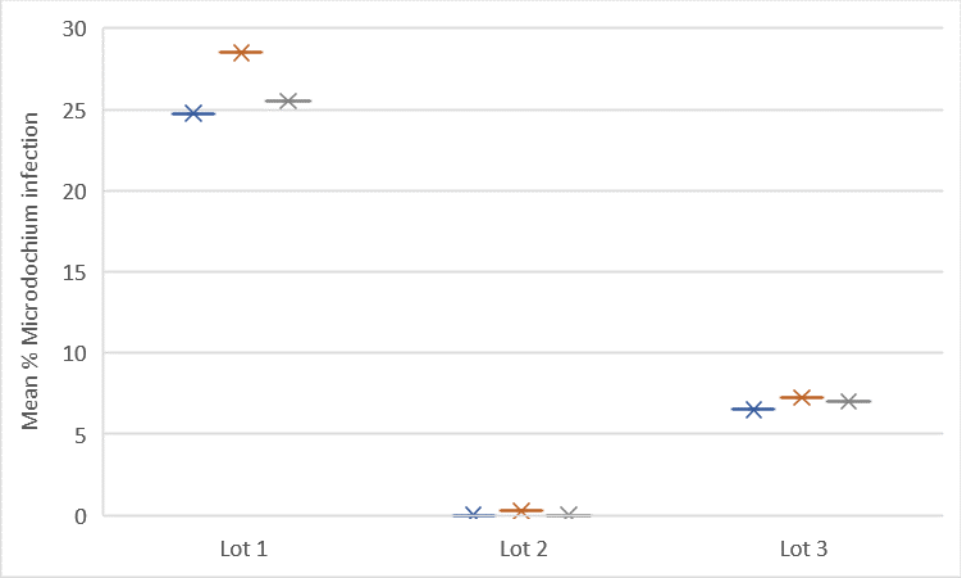


Figure 4: Box and whisker plot for the stability sub-sample mean results for Lots 1, 2 & 3

1.5.1 Healthy seed lot

MS Excel Hampels Outlier Test Lot 2			Healthy			
Lab	Lab Values (Xi)	| Xi - M |	Status			
1	0.00	0.000	OK		**Median (M):**	**0.000**
2	0.25	0.250	Outlier		**MAD:**	**0.000**
3	0.00	0.000	OK		**5.2 X MAD**	**0.000**

The healthy seed lot stability data was added to the homogeneity data to ensure that the new stability test results had not deviated more over time. In the table below S indicates the stability test and H the homogeneity test.

MS Excel Hampels Outlier Test Lot 2			Healthy			
Lab	Lab Values (Xi)	| Xi - M |	Status			
1	0.00	0.000	OK	S	**Median (M):**	**0.000**
2	0.25	0.250	Outlier	S	**MAD:**	**0.000**
3	0.00	0.000	OK	S	**5.2 X MAD**	**0.000**
4	0.25	0.250	Outlier	H		
5	0.25	0.250	Outlier	H		
6	0.00	0.000	OK	H		
7	0.00	0.000	OK	H		
8	0.00	0.000	OK	H		
9	0.00	0.000	OK	H		
10	0.00	0.000	OK	H		
11	0.00	0.000	OK	H		
12	0.00	0.000	OK	H		
13	0.00	0.000	OK	H		

As before the presence of one isolate of *Microdochium* species (although recognized as an outlier by Hampels Tool) is acceptable for the purposes of this test.

1.5.2 Medium infection seed lot

MS Excel Hampels Outlier Test Lot 3			Medium			
Lab	Lab Values (Xi)	| Xi - M |	Status			
1	6.50	0.500	OK		**Median (M):**	**7.000**
2	7.25	0.250	OK		**MAD:**	**0.250**
3	7.00	0.000	OK		**5.2 X MAD**	**1.300**

The medium infection seed lot stability data was added to the homogeneity data to ensure that the new stability test results had not deviated and become outliers over time. In the table below S indicates the stability test and H the homogeneity test.

MS Excel Hampels Outlier Test Lot 3			Medium			
Lab	Lab Values (Xi)	| Xi - M |	Status			
1	6.50	2.000	OK	S	**Median (M):**	**8.500**
2	7.25	1.250	OK	S	**MAD:**	**0.750**
3	7.00	1.500	OK	S	**5.2 X MAD**	**3.900**
4	9.00	0.500	OK	H		
5	9.25	0.750	OK	H		
6	11.00	2.500	OK	H		
7	9.25	0.750	OK	H		
8	9.50	1.000	OK	H		
9	8.25	0.250	OK	H		
10	8.25	0.250	OK	H		
11	8.25	0.250	OK	H		
12	12.50	4.000	Outlier	H		
13	8.50	0.000	OK	H		

Sub-sample 12 (previously homogeneity sub-sample 9) which showed high during the homogeneity test, when combined with the stability test results has now been identified as an outlier.

1.5.3 High infection seed lot

MS Excel Hampels Outlier Test Lot 1			High			
Lab	Lab Values (Xi)	| Xi - M |	Status			
1	24.75	0.750	OK		**Median (M):**	**25.500**
2	28.50	3.000	OK		**MAD:**	**0.750**
3	25.50	0.000	OK		**5.2 X MAD**	**3.900**

The high infection seed lot stability data was added to the homogeneity data to ensure that the new stability test results had not deviated and become outliers over time. In the table below S indicates the stability test and H the homogeneity test.

MS Excel Hampels Outlier Test Lot 1			High			
Lab	Lab Values (Xi)	| Xi - M |	Status			
1	24.75	7.250	OK	S	**Median (M):**	**32.000**
2	28.50	3.500	OK	S	**MAD:**	**2.500**
3	25.50	6.500	OK	S	**5.2 X MAD**	**13.000**
4	32.50	0.500	OK	H		
5	36.25	4.250	OK	H		
6	34.25	2.250	OK	H		
7	32.00	0.000	OK	H		
8	34.25	2.250	OK	H		
9	33.50	1.500	OK	H		
10	32.75	0.750	OK	H		
11	27.75	4.250	OK	H		
12	29.50	2.500	OK	H		
13	29.50	2.500	OK	H		

For Lot 3, point value 12.5 has become an outlier when the homogeneity results are combined with the stability results. See Figure 5 below.

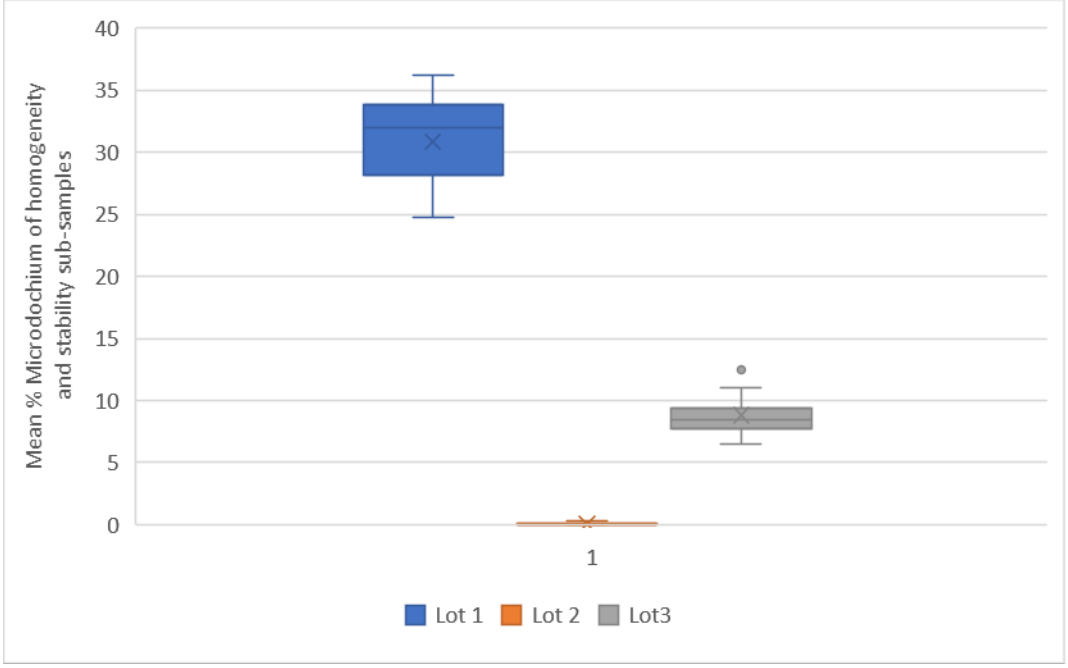


Figure 5: Mean % *Microdochium* of sub-samples for homogeneity and stability tests combined

2 PROFICIENCY TEST RESULTS

2.1 Statistical analysis of data

Results received from participating labs were examined. Raw data for lab replicates is in Appendix A, part 2.

For each lab, the mean replicate results were compared to the allowed tolerances in ISTA Rules (Table 5b, part 1: 2-way test at the 2.5% significance level) and maximum differences between replicates compared to this: for Lab 1, Lot

1 the maximum difference matched the maximum tolerance of 17 for the mean replicate result. Mean result: 28%, Maximum tolerance allowed: 17.

The median result was calculated for each seed lot using the results from the five accredited labs, and this was used as a standard to compare the individual median results to the tolerance given in Miles (1963) Table G8: 5% probability for 400 seed tests in different laboratories. Used to compare results against a standard (median from accredited labs).

Raw data and analysis of accredited labs is given in Appendix A, part 4 and for voluntary labs it is given in Appendix A, part 5.

All accredited labs were within the tolerance given by the median result, although one accredited lab (3) was at the limit of this tolerance for lot 3.

For Lot 1, all voluntary labs were within the tolerance given by the median results for the accredited labs.

For Lot 2, one voluntary lab (7) was outside the tolerance given by the median result for the accredited labs, accounting for the allowed presence of one single colony of *Microdochium* species in 400 seeds.

For Lot 3, all voluntary labs were within the tolerance given by the median result for the accredited labs.

Table 3: Allowed deviation from the standard (median) for each seed lot

Lot	Accredited lab Median % infection	Tolerance: allowed % deviation from the standard (median) for 400 seeds
1	28	6
2	0	0.25
3	8	4

2.2 Analysis of data

2.2.1 Quantitative results

Table 4: All Participating Labs Mean replicate results for Lots 1, 2 and 3 (Raw data in Appendix A, part 2).

		High	Healthy	Medium
Accredited	Lab code	1	2	3
Y	1	28.25	0.00	8.75
Y	2	28.00	0.00	6.75
Y	3	25.25	0.00	6.50
Y	4	28.25	0.00	8.25
N	5	30.75	0.25	10.00
N	6	28.25	0.00	6.25
N	7	32.25	2.75	11.00
Y	8	30.00	0.00	7.75
N	9	23.75	0.00	3.75

2.2.1.1 Results of statistical tools used: healthy level; medium level; high level

Hamples Outlier Test

Healthy

Pathogen	Hamples Outliers Test
Microdochium nivale and *M. majus*	

MS Excel Hampels Outlier Test Lot 2 Healthy Including outlier

Lab	Lab Values (Xi)	| Xi - M |	Status
1	0.00	0.000	OK
2	0.00	0.000	OK
3	0.00	0.000	OK
4	0.00	0.000	OK
5	0.25	0.250	Outlier
6	0.00	0.000	OK
7	2.75	2.750	Outlier
8	0.00	0.000	OK
9	0.00	0.000	OK

Median (M):	**0.000**
MAD:	**0.000**
5.2 X MAD	**0.000**

MS Excel Hampels Outlier Test Lot 2 Healthy Excluding outlier

Lab	Lab Values (Xi)	| Xi - M |	Status
1	0.00	0.000	OK
2	0.00	0.000	OK
3	0.00	0.000	OK
4	0.00	0.000	OK
5	0.25	0.250	Outlier
6	0.00	0.000	OK
8	0.00	0.000	OK
9	0.00	0.000	OK

Median (M):	**0.000**
MAD:	**0.000**
5.2 X MAD	**0.000**
SD	0.088388

Medium infection

Pathogen	Hamples Outliers Test
Microdochium nivale and *M. majus*	

MS Excel Hampels Outlier Test Lot 3 Medium

Lab	Lab Values (Xi)	| Xi - M|	Status		
1	8.75	1.000	OK	**Median (M):**	**7.750**
2	6.75	1.000	OK	**MAD:**	**1.500**
3	3.50	4.250	OK	**5.2 X MAD**	**7.800**
4	8.25	0.500	OK		
5	10.00	2.250	OK		
6	6.25	1.500	OK	**SD**	2.418103
7	11.00	3.250	OK		
8	7.75	0.000	OK		
9	3.75	4.000	OK		

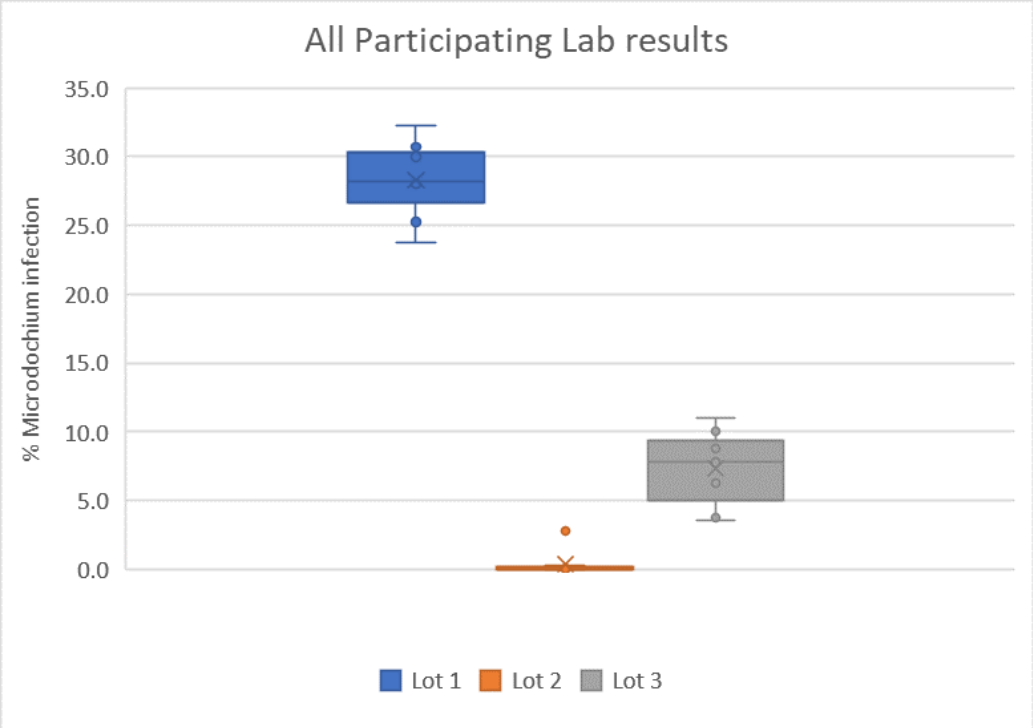
High infection

Pathogen	Hamples Outliers Test
Microdochium nivale and *M. majus*	

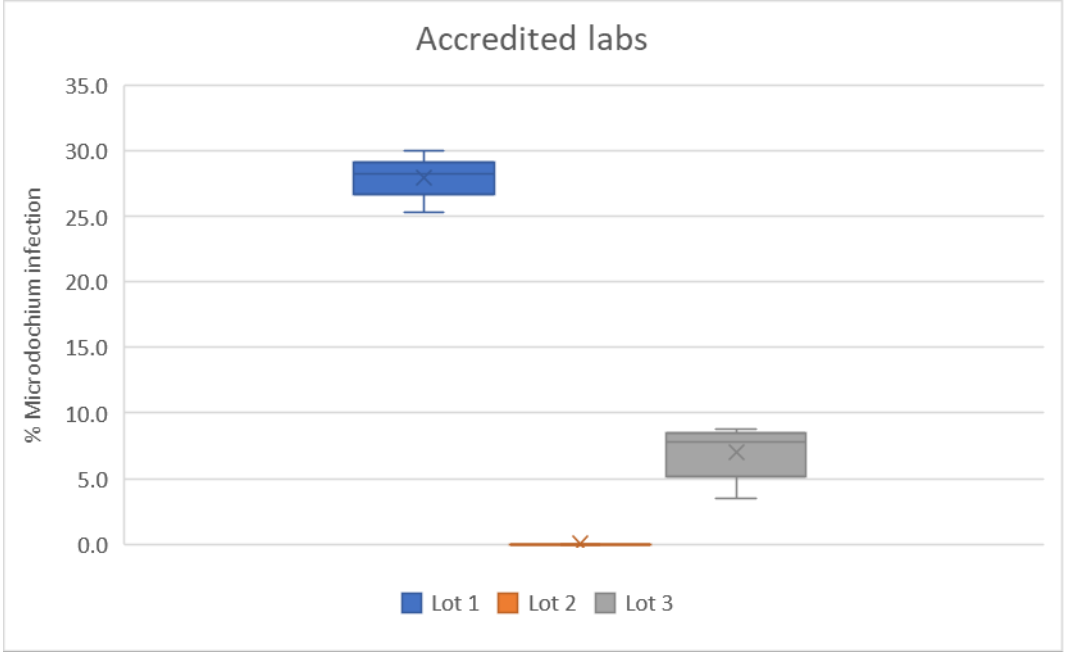
MS Excel Hampels Outlier Test Lot 1 High

Lab	Lab Values (Xi)	| Xi - M|	Status		
1	28.25	0.000	OK	**Median (M):**	**28.250**
2	28.00	0.250	OK	**MAD:**	**1.750**
3	25.25	3.000	OK	**5.2 X MAD**	**9.100**
4	28.25	0.000	OK		
5	30.75	2.500	OK		
6	28.25	0.000	OK	**SD**	1.809
7	32.25	4.000	OK		
8	30.00	1.750	OK		
9	23.75	4.500	OK		

Box Plot of Participating Lab Results



Comparing all participating labs, one clear outlier was observed in Lot 2 (Healthy) and there was some variation observed around the median result.



Comparing only labs with ISTA accreditation for validated SH method 7-022 no labs showed divergent results.

2.2.1.2 Rating system and computations of laboratories

Z-scores for each participating lab were calculated by subtracting the lab result from the mean results obtained from all accredited labs and dividing this by the standard deviation of all lab results for each lot.

Participating lab z scores:

Lab code	High Z score	Medium Z score	Healthy Z score
1	0.122	0.724	0.000
2	0.020	-0.103	0.000
3	-1.096	-1.447	0.000
4	0.122	0.517	0.000
5	1.137	1.241	0.291
6	0.122	-0.310	0.000
7	1.746	1.654	3.205
8	0.832	0.310	0.000
9	-1.705	-1.344	0.000

Rating z score values:

Rating	Z score
A	≤0.67
B	>0.67 ≤1.5
C	<2.33
BMP	>2.33

Lab Rating sheet

	Max abs (z score) for the healthy lot (2)			Max abs (z score) for the medium lot (3)			Max abs (z score) for the high lot (1)				
Minimum requirements for A rating:	0.67		and	0.67		and	0.67				
Minimum requirements for B rating:	1.5		and	1.5		and	1.5				
Minimum requirements for C rating:	2.33		and	2.33		and	2.33				
Lab	Absolute (z score) for the healthy lot	Rating		Absolute (z score) for the medium lot	Rating		Absolute (z score) for the high lot	Rating		Overall Rating (A, B, C or BMP)	BMP: Below minimal performance
1	0.000	A		0.724	B		0.122	A		B	>2.33
2	0.000	A		-0.103	A		0.020	A		A	
3	0.000	A		-1.447	B		-1.096	B		B	
4	0.000	A		0.517	A		0.122	A		A	
5	0.291	A		1.241	B		1.137	B		B	
6	0.000	A		-0.310	A		0.122	A		A	
7	3.205	BMP		1.654	C		1.746	C		BMP	
8	0.000	A		0.310	A		0.832	B		B	
9	0.000	A		-1.344	B		-1.705	C		C	

3 Method declared by participants

All participating labs used ISTA validated SH Method 7-022.

There were differences noted in the type of media used, either Potato Dextrose Agar (PDA) or Malt Agar (MA), and also in the manufacturer of the media used by participating labs. No labs used a type of media that was not recommended in ISTA SH method 7-022.

Four participating labs incubated in darkness with no light, but some labs also used near-ultraviolet light (NUV) for a period to enable the typical salmon pink colour to develop on the *Microdochium* colonies. The use of near-ultraviolet

light can assist with identification. Only one accredited lab indicated the use of NUV, whereas three out of four voluntary labs indicated that NUV was used.

Table 5: Additional information provided by participating labs

Lab No	Accredited	Media	Light
1	Yes	PDA (Merck)	None
2	Yes	MA (in-house)	None
3	Yes	Replicate 1 & 2 on PDA, replicate 3 & 4 on MA (both Merck)	No comment
4	Yes	PDA (Oxoid)	None
5	No	Neogen	None, 3 hours NUV at end of test
6	No	PDA (Merck)	7 days in the dark, 24 h NUV
7	No	PDA (Difco)	None
8	Yes	PDA (Difco)	Dark + NUV
9	No	MA (Applichem)	7 days in the dark, 24 h NUV

4 CONCLUSION

All laboratories with existing accreditation for ISTA validated seed health method 7-022 achieved acceptable Z scores.

Only one of the four voluntary laboratories achieved a BMP marking.

Median results for each seed lot were calculated using the results from the five participating labs that are ISTA accredited for this method. Standard errors are based on the assumption that data is binomial – since there is no evidence to the contrary in this data set. No heterogeneity was observed for any of the three seed lots in the homogeneity pre-test, although one replicate of the homogeneity test for the medium seed lot became an outlier once stability test results were added. There were no differences between the five accredited labs, however non-accredited voluntary participants showed more variation.

The media used may account for some of the variation seen, this is likely to be due to different manufacturers of media using varying amounts of constituents. Lab 3 whilst being accredited for this method saw differences between the Potato Dextrose Agar (PDA) and Malt Agar (MA) used (splitting their results over two different media types), however the combined result of the results still fell within the acceptable tolerances, but the MA results for Lot 3 showed only one colony on the two replicates which was unexpected but not outside the replicate tolerance. The low isolate number may have been due to sample variation, but no results as low as this were observed from any of the homogeneity or stability tests, neither did any other participating lab record results as low as this. There is the possibility that the Malt Agar used was responsible for the low *Microdochium* colony numbers observed.

For a test for the purposes of an Orange International Certificate only one type of media should be used for the whole test.

Lab 7 incorrectly identified colonies on Lot 2 (healthy) as *Microdochium* species, giving an unexpectedly high result and therefore the false positive results merited the BMP rating.

The use of near-ultraviolet light is helpful for laboratories that are less familiar with the identification of *Microdochium* spp.

5. REFERENCES

Miles, S.R. (1963), “Handbook of Tolerances and Measures of Precision for Seed Testing”, Proceedings of the International Seed Testing Association, Vol. 28, No. 3, 525-686

Current International Seed Testing Association Rules, Chapter 5, Table 5b.

International Seed Testing Association document PT-P-03: “Organizing and Analyzing Results of the Seed Health Proficiency Tests”, version 3.0

International Seed Testing Association document PT-P-01: “ISTA Standard Proficiency Test”, version 5.0

Appendix A:

1) Raw data for homogeneity test

Microdochium PT22 SH Results Homogeneity																	
		Lot 1	Date: 3/5/22				Lot 2	Date: 4/5/22				Lot 3	Date: 5/5/22				
Lab	No	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	Media
SASA	1	35	30	31	34	32.5	1	0	0	0	0.25	12	13	6	5	9	PDA (Oxoid)
SASA	2	32	35	45	33	36.25	1	0	0	0	0.25	10	10	9	8	9.25	PDA (Oxoid)
SASA	3	35	37	38	27	34.25	0	0	0	0	0	13	9	10	7	11	PDA (Oxoid)
SASA	4	35	29	29	35	32	0	0	0	0	0	10	11	7	9	9.25	PDA (Oxoid)
SASA	5	31	37	30	39	34.25	0	0	0	0	0	9	12	8	9	9.5	PDA (Oxoid)
SASA	6	28	34	32	40	33.5	0	0	0	0	0	5	10	6	12	8.25	PDA (Oxoid)
SASA	7	37	34	33	27	32.75	0	0	0	0	0	5	9	11	8	8.25	PDA (Oxoid)
SASA	8	32	22	29	28	27.75	0	0	0	0	0	9	6	9	9	8.25	PDA (Oxoid)
SASA	9	27	30	30	31	29.5	0	0	0	0	0	14	11	11	14	12.5	PDA (Oxoid)
SASA	10	35	27	22	34	29.5	0	0	0	0	0	10	6	13	5	8.5	PDA (Oxoid)
					Average	32.23				Average	0.05				Average	9.38	
					Std dev	2.610				Std dev	0.105				Std dev	1.381	
					Max	36.25				Max	0.25				Max	12.50	
					Min	27.75				Min	0.00				Min	8.25	

2) Raw data for the lab test results

Microdochium PT22 SH Results																
		Lot 1	High				Lot 2	Healthy				Lot 3	Medium			
Lab No	Accredited	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
1	Yes	22	38	21	32	28.25	0	0	0	0	0.00	9	7	7	12	8.75
2	Yes	26	23	28	35	28	0	0	0	0	0.00	6	5	10	6	6.75
3	Yes	20	21	30	30	25.25	0	0	0	0	0.00	6	7	0	1	6.50
4	Yes	28	25	28	32	28.25	0	0	0	0	0.00	9	9	10	5	8.25
5	No	31	32	29	31	30.75	1	0	0	0	0.25	16	7	8	9	10.00
6	No	28	26	35	24	28.25	0	0	0	0	0.00	4	7	6	8	6.25
7	No	36	25	37	31	32.25	3	3	2	3	2.75	9	7	14	14	11.00
8	Yes	34	24	32	30	30.00	0	0	0	0	0.00	11	6	7	7	7.75
9	No	25	25	24	21	23.75	0	0	0	0	0.00	5	3	3	4	3.75

3) Raw data for the stability test

Microdochium PT22 SH Stability Results																	
		Lot 1	Date: 22/9/22				Lot 2	DATE: 22/9/2022				Lot 3	Date: 23/9/22				
Lab	No	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	Media
SASA	1	25	27	22	25	24.75	0	0	0	0	0	3	8	6	9	6.5	PDA (Oxoid)
SASA	2	26	31	28	29	28.5	0	0	0	1	0.25	8	9	5	7	7.25	PDA (Oxoid)
SASA	3	27	23	26	26	25.5	0	0	0	0	0	6	8	10	4	7	PDA (Oxoid)
					Average	26.25				Average	0.08				Average	6.92	
					Std dev	1.984				Std dev	0.144				Std dev	0.382	
					Max	28.50				Max	0.25				Max	7.25	
					Min	24.75				Min	0.00				Min	6.50	

4) Raw data for the accredited lab test analysis, comparison of replicate tolerances and comparison with accredited lab median result as a standard

		Lot 1	High infection							
Lab No	Accredited	1	2	3	4	Mean	Replicate tolerance: Table 5B, Part 1, 4 replicates of 100 seeds.	Actual replicate difference	Tolerance against standard median of 28.25, 5% probability for 400 seed tests in different laboratories, Miles (1963) Table G8	Actual difference from median	
1	Yes	22	38	21	32	28.25	17	17	7	0	
2	Yes	26	23	28	35	28.00	17	12	7	0	
3	Yes	20	21	30	30	25.25	17	10	7	3	
4	Yes	28	25	28	32	28.25	17	7	7	0	
8	Yes	34	24	32	30	30.00	18	10	7	2	
					Mean	27.95					
					Median	28.25					
Replicates for all accredited laboratories were in tolerance.											
All accredited labs were in in tolerance compared to the median standard.											
		Lot 2	Healthy							
Lab No	Accredited	1	2	3	4	Mean	Replicate tolerance: 4 replicates of 100 seeds, homogeneity results 1 acceptable	Actual replicate difference	Tolerance against standard median of Nil, for 400 seed tests in different laboratories,	Actual difference from Nil	
1	Yes	0	0	0	0	0	1	0	1	0	
2	Yes	0	0	0	0	0	1	0	1	0	
3	Yes	0	0	0	0	0	1	0	1	0	
4	Yes	0	0	0	0	0	1	0	1	0	
8	Yes	0	0	0	0	0	1	0	1	0	
					Mean	0					
					Median	0					
All accredited labs replicates were Nil											
All accredited labs were in tolerance with the allowed variation of 1.											
		Lot 3	Medium infection							
Lab No	Accredited	1	2	3	4	Mean	Replicate tolerance: Table 5B, Part 1, 4 replicates of 100 seeds.	Actual replicate difference	Tolerance against standard median of 8, 5% probability for 400 seed tests in different laboratories, Miles (1963) Table G8	Actual difference from median	
1	Yes	9	7	7	12	8.75	11	5	4	1	
2	Yes	6	5	10	6	6.75	10	5	4	1	
3	Yes	6	7	0	1	3.50	7	7	4	4	
4	Yes	9	9	10	5	8.25	10	5	4	0.5	
8	Yes	11	6	7	7	7.75	10	5	4	0	
					Mean	7.00					
					Median	7.75					
All accredited lab replicates were within tolerance.											
All accredited labs were within tolerance of the median result as a standard. Lab 3 showed the greatest deviation from the mean for lot 1 and 3, and for Lot 3 is at the limit of variation allowed.											
Lab 3 tested replicates 1 & 2 on Merck PDA, and replicates 3 & 4 on Merck MA, the low results from MA were unusual compared to all the other test results.											

5) Raw data for the voluntary lab test analysis, comparison of replicate tolerances and comparison with accredited lab median result as a standard

		Lot 1	High infection							
Lab No	Accredite	1	2	3	4	Mean	Replicate tolerance: Table 5B, Part 1, 4 replicates of 100 seeds.	Actual difference	Tolerance against standard median of 28, 5% probability for 400 seed tests in different laboratories, Miles (1963) Table G8	Actual difference from median
5	No	31	32	29	31	30.75	16	3	6	3
6	No	28	26	35	24	28.25	16	11	6	0
7	No	36	25	37	31	32.25	17	13	6	4
9	No	25	25	24	21	23.75	15	4	6	4
					Mean	28.75				
					Median	29.50				
			Accredited labs median			28.25				
	All replicate results of voluntary labs are in tolerance.									
	All voluntary labs are in tolerance of the median set by the accredited lab results as a standard.									
		Lot 2	Healthy							
Lab No	Accredite	1	2	3	4	Mean	Replicate tolerance: 4 replicates of 100 seeds, homogeneity results 1 acceptable	Actual difference from accredited labs median	Tolerance against standard median of Nil, for 400 seed tests in different laboratories, homogeneity results 1 acceptable	Actual difference from 1
5	No	1	0	0	0	0.25	1	1	1	0
6	No	0	0	0	0	0	1	0	1	0
7	No	3	3	2	3	2.75	1	3	1	2
9	No	0	0	0	0	0	1	0	1	0
					Mean	0.75				
					Median	0.13				
			Accredited labs median			0				
	All replicate results of voluntary labs are in tolerance.									
	3 out of 4 voluntary labs are in tolerance of the median set by the accredited lab results as a standard. One lab is outside that tolerance, lab 7.									
		Lot 3	Medium infection							
Lab No	Accredite	1	2	3	4	Mean	Replicate tolerance: Table 5B, Part 1, 4 replicates of 100 seeds.	Actual difference	Tolerance against standard median of 8, 5% probability for 400 seed tests in different laboratories, Miles (1963) Table G8	Actual difference from median
5	No	16	7	8	9	10.00	10	9	4	2
6	No	4	7	6	8	6.25	8	4	4	2
7	No	9	7	14	14	11.00	11	7	4	3
9	No	5	3	3	4	3.75	6	2	4	2
					Mean	7.75				
					Median	8.13				
			Accredited labs median			7.75				
	All replicate results of voluntary labs are in tolerance.									
	All voluntary labs are in tolerance of the median set by the accredited lab results as a standard.									

6. Calculation of Lab z scores

Z score calculation based on the mean result of accredited labs and standard deviation of population									
				Lot 1	High	Lot 2	Healthy	Lot 3	Medium
			Lab code	Mean	Z score	Mean	Z score	Mean	Z score
		O	1	28.25	0.122	0.00	0.000	8.75	0.724
		O	2	28.00	0.020	0.00	0.000	6.75	-0.103
		O	3	25.25	-1.096	0.00	0.000	3.50	-1.447
		O	4	28.25	0.122	0.00	0.000	8.25	0.517
		O	8	30.00	0.832	0.00	0.000	7.75	0.310
		V	5	30.75	1.137	0.25	0.291	10.00	1.241
		V	6	28.25	0.122	0.00	0.000	6.25	-0.310
		V	7	32.25	1.746	2.75	3.205	11.00	1.654
		V	9	23.75	-1.705	0.00	0.000	3.75	-1.344
Mean of accredited labs				27.95		0.00		7.00	
Median of accredited labs				28.25		0.00		7.75	
Standard deviation of population				2.463		0.858		2.418	