Detection of Cucumber green mottle mosaic virus (CGMMV), Melon necrotic spot virus (MNSV) and Squash mosaic virus (SqMV) in Cucurbit Seeds by ELISA

Validation report, June 2025

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ISHI VALIDATION REPORTS

This ISHI validation study has been conducted to determine the fitness of the described method for its intended purpose according to the ISHI Guidelines for the Validation of Seed Health Methods[1] and followed by an independent review of its outcome.

DISCLAIMER

ISF cannot guarantee that labs following the protocol described herewith will obtain similar results. Many factors, such as staff skills, laboratory equipment and conditions, reagents and sampling methods can influence the results. Consequently, in case of any litigation, ISF will not accept any liability on the use of these tests.

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SUMMARY

Cucumber green mottle mosaic virus (CGMMV, now *Tobamovirus viridimaculae*), Melon necrotic spot virus (MNSV, now *Gammacarmovirus melonis*) and Squash mosaic virus (SqMV, now *Comovirus cucurbitae*) are seed borne viruses that are known to cause a wide range of symptoms on cucurbit crops. The Enzyme-Linked Immunosorbent Assay (ELISA) has been commonly used by the seed industry for many years for the detection of the viruses on seeds and is considered as a reliable tool for preventing the distribution of infected seeds to the market. The aim of this project is to validate the ELISA assay for the detection of CGMMV, MNSV and SqMV in seeds, according the ISHI validation guidelines. ELISA is currently the only available method for the detection of CGMMV and MNSV, while for SqMV, the ELISA is a pre-screen assay that is followed by a grow-out assay.

In this validation study, the performance criteria analytical specificity, analytical sensitivity, selectivity, repeatability, reproducibility, and diagnostic performance were validated to determine if the ELISA for CGMMV, MNSV and SqMV is fit for purpose.

For the analytical specificity assessment, 29 CGMMV target strains, 15 MNSV target strains and 12 SqMV target strains were tested by ELISA. The strains tested were distinguished by crop, production origin and year to cover a wide range of isolate types per virus. All target strains were detected correctly by ELISA showing a good inclusivity for the ELISA protocol. For the non-targets, 26 strains were tested, representing at least seven virus species that attack cucurbits, isolated from different crops, production origin and years. In addition, CGMMV, MNSV and SqMV strains double functioned as non-target strains for the other viruses. All non-target strains were not detected by ELISA, which showed a good exclusivity of the ELISA.

In the analytical sensitivity, the LOD was determined by testing five different crops in different infection levels equivalent to half, one, two or three infected seeds out of a subsample of 100 seeds total. With a confidence of >95%, the LOD for the detection of CGMMV, MNSV and SqMV by ELISA was determined to be at least one seed out of 100 seeds.

CGMMV, MNSV and SqMV were each detected in 20 different seed matrices by ELISA, showing a good selectivity of the assay. The ELISA results of all three viruses were repeatable, as shown during the evaluation of selectivity, with a 100% accordance.

Two comparative tests (CT), for the assessment of CGMMV and then MNSV and SqMV, were performed among six and seven laboratories, respectively. The concordance percentage of the six participating laboratories in CGMMV CT was above 95.8% for the different infection levels. In the MNSV and SqMV CT results, out of the seven participating laboratories, one of them obtained poor performance with the positive control used in the test and results were deemed inconclusive. This resulted in excluding the laboratory from the concordance estimation. The concordance rate of the remaining six participating laboratories in the MNSV and SqMV CT was >97.1% for the different infection levels. The concordance results of both CTs are above the required 90%, which indicates that the reproducibility criterion was met.

The diagnostic performance of the ELISA assay was evaluated based on the CT results for CGMMV, MNSV and SqMV. Due to a decrease in infection level in the medium infected CGMMV subsamples, demonstrated in the stability test done after performing the CT, these subsamples were not included in the diagnostic sensitivity and specificity calculations. The actual detected samples were compared to the calculated probabilities, which showed that results fell within the expected range. With a diagnostic sensitivity of 100%, 100% and 100% and a diagnostic specificity of 97.9%, 98.5% and 100% for CGMMV, MNSV and SqMV, respectively. The diagnostic sensitivity and diagnostic specificity of all three viruses were thus all above the requirement of 95% for this criterion.

This validation report confirms that the ELISA assay for the detection of CGMMV, MNSV and SqMV is able to detect one seed in a background of 100 seeds. The ISHI CGMMV, MNSV and SqMV ELISA assay is fit for purpose. When performing the assay, it is strongly recommended to follow the ISHI best practices for ELISA.

1. INTRODUCTION

Cucumber green mottle mosaic virus (CGMMV) also known as *Tobamovirus viridimaculae*, Melon necrotic spot virus (MNSV) also known as *Gammacarmovirus melonis* and Squash mosaic virus (SqMV) also known as *Comovirus cucurbitae,* are seed borne viruses that can attack cucurbit crops during plant growth and can cause damages to yield, which can in turn lead to economic losses. Their detection in seeds of cucurbit species is an important tool for disease control.

CGMMV, which belongs in the *Tobamovirus* genus of the *Virgaviridae* family, is a relatively stable virus that can survive for a long period of time. The virus can cause symptoms of mottling and mosaic on the leaves and mottling to the fruit. Several outbreaks of CGMMV around the world were reported in the last decade (Reingold *et. al*, 2015). MNSV is a *Carmovirus* of the *Tombusviridae* family. MNSV is transmitted by the *Olpidium* fungus, mechanically or by seeds. The virus can cause necrotic spots and necrotic lesions on the leaves and stem of the plant (Sugiyama and Sakata, 2004). SqMV is a *Comovirus* of the *Comoviridae* family. SqMV can cause symptoms of mosaic and distortion on the leaves and deformation and color change of the fruits on severe infection (Şevik and Toksöz, 2008).

Enzyme-linked Immunosorbent Assay (ELISA) is a serological method that has been used for many years for the detection of different viruses and bacteria. Double Antibody Sandwich ELISA (DAS-ELISA) is based on sandwich structure of a primary antibody, an antigen (of the tested pathogen), and a secondary antibody. The antibodies are specific for the tested pathogen, while the secondary antibody is also linked to a reporter enzyme to allow its visualisation.

In 2005, ISHI laboratories participated in an international comparative test to evaluate the use of DAS-ELISA for the detection of these viruses. This method (ista-rules-2025-shmethods-7-026-final.pdf) is routinely used by seed companies, and several proficiency tests conducted during the last years have demonstrated its robustness. If no virus is detected, the seed lot is considered healthy. However, a positive DAS-ELISA is only indicative of the presence of virus antigens and gives no information on whether the virus is infectious. For SqMV, a positive pre-screen ELISA is followed by a confirmation method based on a grow-out and DAS-ELISA assay. Figure 1 presents the method process workflow for CGMMV, MNSV and Figure 2, the method process workflow for SqMV.

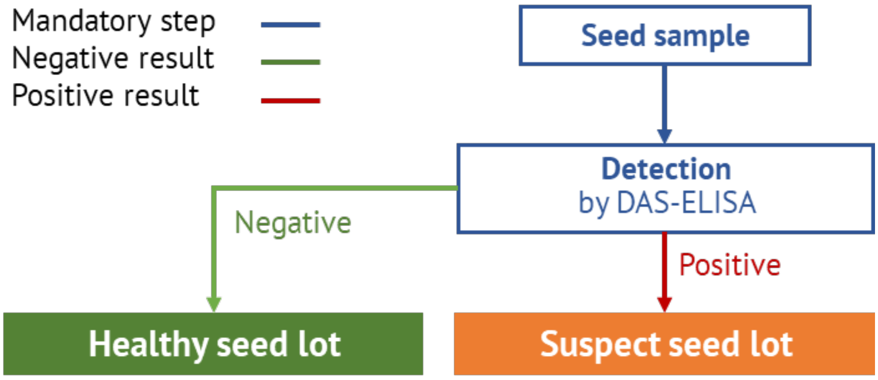


Figure 1. Method process workflow for CGMMV, MNSV.

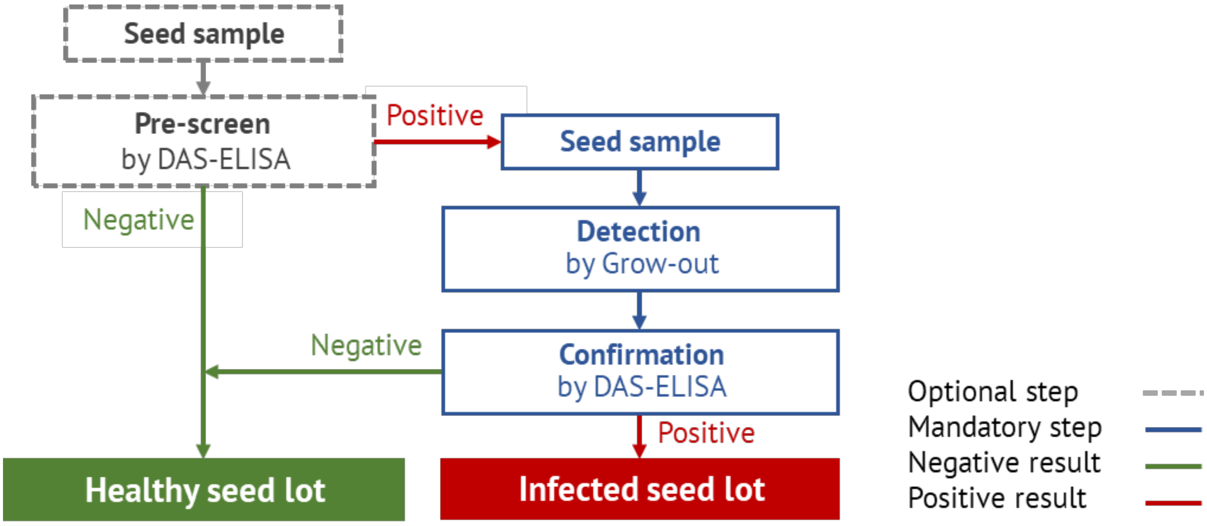


Figure 2. Method process workflow for SqMV.

With recent disease outbreaks in USA and Australia, CGMMV, MNSV and SqMV are increasingly regulated worldwide. ISHI re-examined the method validation data generated in 2005 (data not published) and concluded that the method should be revalidated to meet the conditions laid out in the current ISHI guidelines for validating seed health tests. As part of the validation, an ISHI protocol for this method was established. All validation experiments were performed based on the version of the method presented in Annex A.

2. OBJECTIVES

This validation report describes the experiments and results to investigate whether the ISHI ELISA method to detect CGMMV, MNSV and SqMV in seeds of cucurbits is fit for purpose. The described experiments assess the analytical specificity, analytical sensitivity, selectivity, repeatability, reproducibility and diagnostic performance of the ELISA and were designed according to the ISHI guidelines for Validation of Seed Health Tests (ISHI, 2020).

3. METHOD VALIDATION

The ELISA protocol for detection of CGMMV, SqMV and MNSV in seeds of cucurbits used in the validation experiments is presented in Annex A.

3.1. Analytical specificity

<u>Definition ISHI guidelines:</u> *The ability of an assay to detect the target(s) pathogens (inclusivity) while excluding non-targets (exclusivity).*

The analytical specificity requirements will be met for each pathogen when the ELISA gives a positive result for the specific pathogen strains tested and a negative result for all non-target strains tested.

<u>Experimental approach</u>

Data were generated from 2018 to 2020 by three companies, using all strains present in the companies at the time of testing. The number of strains tested for determining inclusivity and exclusivity for the independent detection of CGMMV, MNSV and SqMV are presented in Table 1. Representative strains from each target pathogen were tested by PCR, followed by sequencing to confirm the identity of the strains. The PCR, for each pathogen, was done with *in house* primers that were tested *in situ*. Sequencing was performed by Sanger sequencing. The non-target strains contained representatives of at least seven viral species, which are known to occur on cucurbits or are related to the target viruses, namely Cucumber fruit mottle mosaic virus (CFMMV), Cucumber mosaic virus (CMV), Cucumber vein yellowing virus (CVYV), Kyuri green mottle mosaic virus (KGMMV), Squash leaf curl virus (SLCV), Watermelon chlorotic stunt virus (WMCSV) and Zucchini yellow mosaic virus (ZYMV). Other viruses such as WGMMV and ZGMMV were not included in this experiment due to the impossibility to acquire strains and/or infected seeds. In addition, CGMMV, MNSV and SqMV strains double functioned as non-target strains for the other viruses.

The ELISA was performed following the protocol described in Annex A and using antibodies purchased from Prime Diagnostics.

Table 1. Number of strains tested for determination of inclusivity and exclusivity.

Strains	Number	Parameter tested
CGMMV	29	Inclusivity
MNSV	15	
SqMV	12	
Non-targets	26	Exclusivity

<u>Results</u>

Results of the ELISA, PCR and sequencing are presented in Annex B.

Inclusivity

The ELISA results for CGMMV, MNSV and SqMV strains, including the positive and negative controls, are shown in Table B.1 in Annex B. For the detection of CGMMV, MNSV and SqMV, all target strains were detected by ELISA.

Exclusivity

The ELISA results for CGMMV, SqMV and MNSV non-target strains, including the positive and negative controls, are shown in Table B.2 in Annex B. For the detection of CGMMV, MNSV and SqMV, all non-target strains were negative by ELISA.

Conclusion

The analytical specificity experiments have shown that the requirements for the ELISA were met and that, for this validation criterion, the detection method is deemed fit for purpose.

3.2. Analytical sensitivity

Definition ISHI guidelines: *Smallest amount of the target pathogen that can be detected i.e., the limit of detection (LOD).*

The analytical sensitivity requirements will be met when the LOD of the ELISA is equal or lower than one infected seed per subsample (100 seeds).

Experimental approach

Powder obtained from cucumber and melon seeds infected with CGMMV or SqMV, respectively, was used to spike CGMMV, SqMV and MNSV negative seeds of watermelon, melon, cucumber, squash and pumpkin in several concentrations: equivalent to about half a seed, one, two, and three seeds. Each concentration was tested in 20 subsamples (four subsamples per crop).

For determining the LOD of MNSV, due to lack of naturally infected seeds, infected leaf material was added to the extraction buffer in quantities calibrated to yield optical density (OD) values comparable to those obtained in previous CGMMV and SqMV experiments. These OD values corresponded to the equivalent viral load of ground infected seed material at weights matching concentration of half a seed, one seed, two seeds, and three seeds. The spiked extraction buffer was added to negative seed powder of watermelon, melon, cucumber, squash and pumpkin. Each concentration was tested in 20 independent subsamples (four subsamples per each crop).

Characteristics of the seed samples used for the determination of analytical sensitivity is presented in Table 2.

Table 2. Seed samples used for the determination of analytical sensitivity.

Seeds	Crop	Lot	Production year	Location
Negative	Watermelon	Or 31	2017	USA
	Melon	Or 28	2014	Israel
	Cucumber	LBH100	2018	Peru
	Squash	Or 29	2007	China
	Pumpkin	LBH70	2012	China
	Pumpkin	LBH78	2014	Israel
CGMMV positive	Cucumber	LBH5	2017	Israel
SqMV positive	Melon	Or17	2015	Israel
MNSV positive	Melon leaves	LBH120	2020	Israel

Results

For CGMMV, the ELISA results, presented in Table 3 and in Annex C, Table C.1, obtained after spiking subsamples of watermelon, melon, cucumber, squash and pumpkin, revealed that the detection rate was 90% when a weight equivalent to half a seed spiked per subsample was infected and rose to 100% when infection level was a weight equivalent to one seed spiked per subsample. Therefore, the LOD for CGMMV is one positive seed out of 100 seeds.

For SqMV, the detection rate obtained by ELISA was 60% with a weight equivalent to half a seed spiked per subsample and it rose to 95% with a weight equivalent to one seed spiked per subsample, with positive results obtained in 19 out of 20 subsamples (Table 4 and Annex C, Table C.2). Therefore, the LOD for SqMV is one positive seed out of 100 seeds.

For MSNV, the lowest concentration equivalent to half a seed gave positive ELISA results in all subsamples and all crop species (Table 5 and Annex C, Table C.3). Detection rate was 100% with the weight equivalent to half a seed per subsample, and this represents the LOD for MNSV.

Table 3. Evaluation of analytical sensitivity for CGMMV by ELISA. #/# represents the number of positive subsamples out of a total number of subsamples tested.

# Spiked Seeds	Watermelon	Melon	Cucumber	Squash	Pumpkin	Total	Detection rate
0.5	4+/4	4+/4	4+/4	2+/4	4+/4	18+/20	90%
1	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%
2	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%
3	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%

Table 4. Evaluation of analytical sensitivity for SqMV by ELISA. #/# represents the number of positive subsamples out of a total number of subsamples tested.

# Spiked Seeds	Watermelon	Melon	Cucumber	Squash	Pumpkin	Total	Detection rate
0.5	3+/4	3+/4	2+/4	2+/4	2+/4	12+/20	60%
1	4+/4	3+/4	4+/4	4+/4	4+/4	19+/20	95%
2	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%
3	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%

Table 5. Evaluation of analytical sensitivity for MNSV by ELISA. #/# represents the number of positive subsamples out of a total number of subsamples tested.

# Spiked Seeds	Watermelon	Melon	Cucumber	Squash	Pumpkin	Total	Detection rate
0.5	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%
1	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%
2	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%
3	4+/4	4+/4	4+/4	4+/4	4+/4	20+/20	100%

Conclusion

The analytical sensitivity results obtained show that the LOD for the detection of CGMMV, SqMV and MNSV in cucurbit seeds is at least one positive seed out of 100 seeds. In conclusion, regarding the analytical sensitivity validation, the ELISA assay is deemed fit for purpose.

3.3. Selectivity

Definition ISHI guidelines: *The effect of different matrices on the ability of the method to detect the target pathogen.*

The selectivity requirements will be met when the three pathogens will be detected by ELISA in all matrices tested, which represent a variety of crop species, location, production years and treatment types.

Experimental approach

Powder obtained from seeds infected with either CGMMV or SqMV and from leaf material infected with MNSV, prepared for the evaluation of analytical sensitivity (Section 3.2), was used to spike subsamples of 100 seeds from known healthy seed lots from 20 different matrices (Table 6). Subsamples were artificially contaminated at two different infection levels above the LOD, using powder obtained from two (low concentration) and three (medium concentration) positive seeds.

The 20 spiked samples were tested individually three times for each pathogen by one technician using the same reagents and equipment. Each sample were tested in technical duplicates, giving rise to a total of six data points for each concentration.

Table 6. Negative seed samples used for the determination of selectivity.

Lot	Crop	Production year	Location	Treatment
OR26	Watermelon	2016	Chile	None
OR31	Watermelon	2017	USA	None
LBH71	Watermelon	2018	Africa	None
LBH92	Watermelon	2015	China	None
OR28	Melon	2014	Israel	None
LBH79	Melon	2018	Africa	None
LBH94	Melon	2018	Thailand	Coated
LBH95	Melon	2011	China	Coated
LBH101	Cucumber	2011	The Netherlands	None
OR22	Cucumber	2012	Israel	None
LBH99	Cucumber	2010	China	None
LBH98	Cucumber	2017	India	None
OR29	Squash	2007	China	None
LBH83	Squash	2018	France	None
LBH81	Squash	2016	France	None
LBH85	Squash	2017	France	None
LBH70	Pumpkin	2012	China	None
LBH77	Pumpkin	2017	Japan	None
OR20	Pumpkin	2010	Israel	None
LBH78	Pumpkin	2014	Israel	None

Results

The average OD values for the three repetitions, tested in duplicate, for all three viruses are presented in Table 7. The raw data is presented in Annex D, Table D.1.

For CGMMV, all samples were detected as positive (i.e., OD above the threshold), except for one of the duplicates for the cucumber samples LBH 99 and LBH 101 (Table D.1). The OD values are close to the calculated threshold, as described in the protocol (OD=0.168, in this experiment). Since the ELISA qualitative result is based on the result of the duplicates, the samples are still considered as positive. Therefore, all samples were detected positive in all matrices.

For SqMV, all matrices tested gave consistent positive results with the exception of sample OR26 with medium concentration only (i.e., equivalent to three infected seeds per subsample, Table 7). Sample OR26 with medium concentration was detected as negative in all repetitions. This is most likely due to human error and not matrix effect since the first concentration, which is a lower concentration, was detected positive. This concentration will therefore be excluded from the analysis of the selectivity.

For MNSV, all samples were detected as positive (i.e., OD above the threshold) (Table 7).

Table 7. Average OD values for selectivity experiment. A red cell indicates a positive ELISA result; a green cell indicates a negative ELISA result. Threshold ELISA OD = 2 × average NC (see Table D.1).

Lot	Crop	Concentration	CGMMV	SqMV	MNSV
OR20	Pumpkin	Low (two + seeds spiked)	0.320	1.216	0.248
		Medium (three + seeds spiked)	0.388	1.180	0.751
OR22	Cucumber	Low (two + seeds spiked)	0.641	1.215	0.294
		Medium (three + seeds spiked)	1.142	1.451	0.996
OR26	Watermelon	Low (two + seeds spiked)	0.416	0.865	0.875
		Medium (three + seeds spiked)	0.406	0.093	1.376
OR28	Melon	Low (two + seeds spiked)	0.324	1.574	0.304
		Medium (three + seeds spiked)	0.479	2.067	1.081
OR29	Squash	Low (two + seeds spiked)	0.308	0.263	0.324
		Medium (three + seeds spiked)	0.340	0.278	0.973
OR31	Watermelon	Low (two + seeds spiked)	0.463	0.961	0.339
		Medium (three + seeds spiked)	0.360	1.249	1.191
LBH70	Pumpkin	Low (two + seeds spiked)	0.373	0.975	0.253
		Medium (three + seeds spiked)	0.450	1.175	1.032
LBH71	Watermelon	Low (two + seeds spiked)	0.499	1.499	0.371
		Medium (three + seeds spiked)	0.295	1.032	1.409
LBH77	Pumpkin	Low (two + seeds spiked)	0.377	1.111	0.235
		Medium (three + seeds spiked)	0.613	1.395	0.810
LBH78	Pumpkin	Low (two + seeds spiked)	0.442	0.990	0.299
		Medium (three + seeds spiked)	0.579	1.800	0.979
LBH79	Melon	Low (two + seeds spiked)	0.275	0.849	0.286
		Medium (three + seeds spiked)	0.401	1.529	1.024
LBH81	Squash	Low (two + seeds spiked)	0.474	1.381	0.209
		Medium (three + seeds spiked)	0.563	1.680	0.927
LBH83	Squash	Low (two + seeds spiked)	0.412	1.108	0.237
		Medium (three + seeds spiked)	0.663	1.314	0.783
LBH85	Squash	Low (two + seeds spiked)	0.355	0.976	0.259
		Medium (three + seeds spiked)	0.508	1.219	1.047
LBH92	Watermelon	Low (two + seeds spiked)	0.521	1.571	0.312

Lot	Crop	Concentration	CGMMV	SqMV	MNSV
		Medium (three + seeds spiked)	0.579	2.190	1.065
LBH94	Melon	Low (two + seeds spiked)	0.285	0.787	0.267
		Medium (three + seeds spiked)	0.344	1.158	1.028
LBH95	Melon	Low (two + seeds spiked)	0.257	0.554	0.156
		Medium (three + seeds spiked)	0.300	0.684	0.523
LBH98	Cucumber	Low (two + seeds spiked)	0.267	0.579	0.353
		Medium (three + seeds spiked)	0.239	0.955	1.054
LBH99	Cucumber	Low (two + seeds spiked)	0.250	0.974	0.270
		Medium (three + seeds spiked)	0.211	1.144	1.006
LBH101	Cucumber	Low (two + seeds spiked)	0.183	0.634	0.305
		Medium (three + seeds spiked)	0.229	0.757	1.044

Conclusion

For the three viruses, all samples were detected as positive in both concentrations in all matrices. No effect was observed due to the matrix of the sample. Regarding the selectivity criterion, the ELISA assay is deemed fit for purpose.

3.4. Repeatability

Definition ISHI guidelines: *Degree of similarity in results of replicates of the same seed lots when the method is performed with minimal variations in a single laboratory.*

The repeatability requirements will be met when the accordance is >90% and all samples yield consistent qualitative results in the three repetitions.

Experimental approach

The same samples used for evaluating selectivity of the ELISA assays (Section 3.3, Table 6) were also used for evaluating repeatability. The 20 samples spiked at a low and medium infection levels and healthy negative controls were tested three times (in three independent ELISA plates) for each pathogen by the same technician and using the same reagents and equipment on the same day. For each repetition, samples were tested in technical duplicates, giving rise to a total of six readings per concentration per sample.

The method of Langton *et al.* (2002) was used to evaluate the accordance (repeatability of data) of the qualitative (positive or negative) results of the ELISA.

Results

Results are presented in Annex D, Table D.1. A summary of the data is presented in Table 8.

For CGMMV, one of the 40 replicates in repetition one, in both infection levels, gave a deviating result, giving rise to an accordance of 98.3% (Table 8). For SqMV, OR26 with medium level of infection was excluded from the analysis, since it is more likely due to human error, as mentioned in the selectivity assessment (Section 3.3). With this exclusion, all three repetitions, for SqMV and MNSV, with the three infection levels, qualitatively gave the same results, resulting in an accordance of 100%. Quantitative differences in OD values were observed but are expected and accepted when performing ELISA in different plates.

Conclusion

The accordance for all three viruses, at all three infection levels tested, was >90%. Therefore, the repeatability requirements for the ELISA are met.

Table 8. Evaluation of repeatability (accordance) for three infection levels of CGMMV, MNSV and SqMV by ELISA. #/# represents the number of positive subsamples out of a total number of duplicates tested.

Isolate	Infection level	Repetition 1	Repetition 2	Repetition 3	Accordance
CGMMV	Healthy	0+/2	0+/2	0+/2	100%
	Low	39+/40	40+/40	40+/40	98.3%
	Medium	39+/40	40+/40	40+/40	98.3%
SqMV	Healthy	0+/2	0+/2	0+/2	100%
	Low	40+/40	40+/40	40+/40	100%
	Medium	38+/38	38+/38	38+/38	100%
MNSV	Healthy	0+/2	0+/2	0+/2	100%
	Low	40+/40	40+/40	40+/40	100%
	Medium	40+/40	40+/40	40+/40	100%

3.5. Reproducibility

Definition ISHI guidelines: *Degree of similarity in results when the method is performed across laboratories with replicates of the same subsamples.*

The reproducibility requirements will be met when the concordance of the test results obtained by the different laboratories on the test set are above the accepted values of 90%.

Experimental approach

Two separate comparative tests (CTs) were performed: one for the detection of CGMMV and one for the detection of SqMV and MNSV. CT plans are presented in Annex E.1 and E.2.

CGMMV CT

For the detection of CGMMV, a CT was organized in ISHI with six participating laboratories. All participants received a total of 28 samples of 100 seeds each. The 28 samples consisted of eight healthy samples (two cucumber, two melon, two squash and two watermelon samples), ten CGMMV medium infected samples (five cucumber and five squash samples), and ten CGMMV high infected samples (five melon and five watermelon samples). The healthy samples double-functioned as negative process control (NPC) and the high infected samples as positive process control (PPC). The samples were randomly coded when sent to the participants. Participants were requested to store samples in a cool and dry environment upon arrival and until processing. Detailed organization of the CT plan are presented in Annex E.1.

All participants processed the seed samples according to the protocol described in Annex A. They were asked to grind separately every sample to prevent cross contamination, to report both qualitative (positive/negative), as well as quantitative (OD values) results for each sample and to inform on the grinding method and antisera used, and to follow the threshold calculation as described in the protocol.

In addition to the work described for reproducibility above, the organizing laboratory of the CT had tested prior to the CT, ten samples of 100 seeds of each of the positive seed lots from the four crop species included (cucumber, melon, squash and watermelon), and at least five samples of 100 seeds of each negative seed lot (cucumber, melon, squash and watermelon) used in the CT, for homogeneity testing. One additional CT set of 28 samples was tested by the organizing laboratory of the CT after receiving the results from all participants, for stability testing. This data provided homogeneity data and stability of the samples.

The analysis of the CT data was done at the qualitative level per sample (positive or negative results). The method of Langton *et al.* (2002) was used to evaluate the concordance (reproducibility of data) of the ELISA.

SqMV & MNSV CT

The CT for MNSV and SqMV was conducted by the Seed Extract RT-qPCR project team as a parallel CT for ELISA and RT-qPCR.

For the detection of MNSV and SqMV, a CT was organized with seven participants. All laboratories received a total of 30 samples of 1,000 seeds each, to a total of 300 subsamples of 100 seeds each, as presented in Table 9. The healthy samples double-functioned as negative process control (NPC) and the high infected samples as positive process control (PPC). The samples were randomly coded when sent to the participants. Participants were requested to store samples in a cool and dry environment upon arrival and until processing. A detailed CT plan is available as Annex E.2.

Table 9. CT samples per infection category.

Infection category	Number of samples
Healthy	18
MNSV	5
SqMV	7

All participants processed the seed samples according to the protocol described in Annex A. They were asked to grind separately every sample to prevent cross contamination, to report both qualitative (positive/negative) as well as quantitative (OD values) results for each sample and to provide information on the grinding method, antisera used, and the threshold calculation.

The analysis of the CT data was done on a qualitative level per sample (positive or negative results). The method of Langton *et al.* (2002) was used to evaluate the concordance (reproducibility of data) of the ELISA.

Results

CGMMV CT

For CGMMV, the homogeneity and stability results, together with a summary of the CT results, are presented in Table 10. The full data of the CT including Stability test are presented in Annex E, Table E.3.

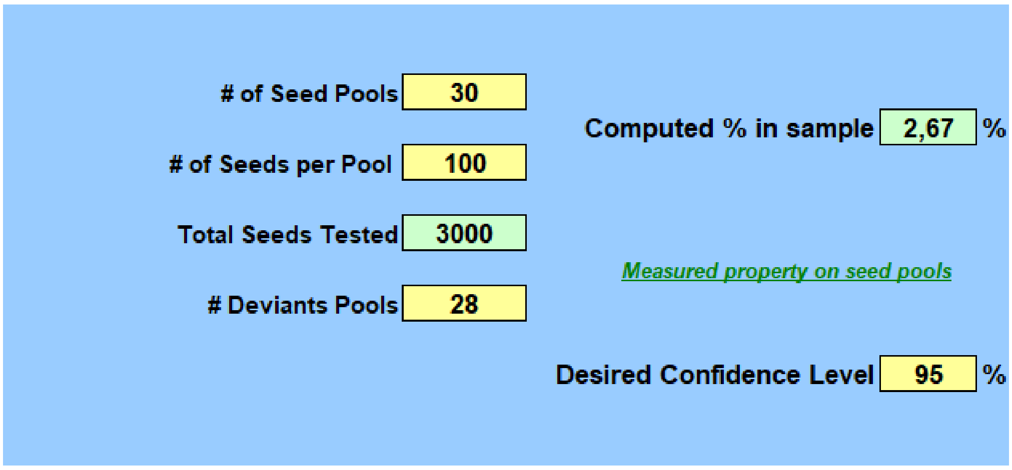
Table 10. Results of homogeneity, CT and stability for CGMMV by ELISA. #/# represents the number of positive samples out of a total number of samples tested.

Sample	Homogeneity results	Stability results	Comparative test results
Healthy	0+/20	0+/8	1+/48
Medium	20+/20	8+/10	53+/60
High	20+/20	10+/10	60+/60

In the homogeneity test, all positive samples, the medium and high infected, tested positive by ELISA and all healthy samples tested negative, indicating that all samples were homogeneous.

In the stability test, all healthy samples resulted in a negative result, and all high infected seed samples in a positive result, corresponding to the results from the homogeneity test. This suggests that the health status of these samples is stable. However, for the medium infected seed lots, not all samples were detected as positive as expected (Table 10). These results suggest that the level of infection decreased with time.

Due to the problems with stability, no concordance can be calculated for the medium infected seed samples. For these samples, the results from the CT should fall within the expected number of infected samples, as calculated based on the average percentage of infection obtained from the homogeneity and stability results. The ratio of infection is calculated using Seedcalc8 (https://www.seedtest.org/en/services-header/tools/statistics-committee/statistical-tools-seed-testing.html, March 2023) as the "computed % in sample" at 95% confidence. The rate of infection of the medium infected seed lots, corresponding to 28 positive samples out of 30 total, was 2.67% (Figure 3). This rate of infection was used for the calculation of the probability to obtain infected samples from the tested samples with "probability of *k* positive samples out of *n*" tool (https://www.seedtest.org/en/services-header/tools/seed-health-committee/seed-health-toolbox.html, March 2023). Considering each value with a probability higher than 5%, the CT should give between eight to 10 positives out of the 10 samples tested in the CT for the medium infection seed lot (Figure 4). The CT results per laboratory are summarized in Table 11.



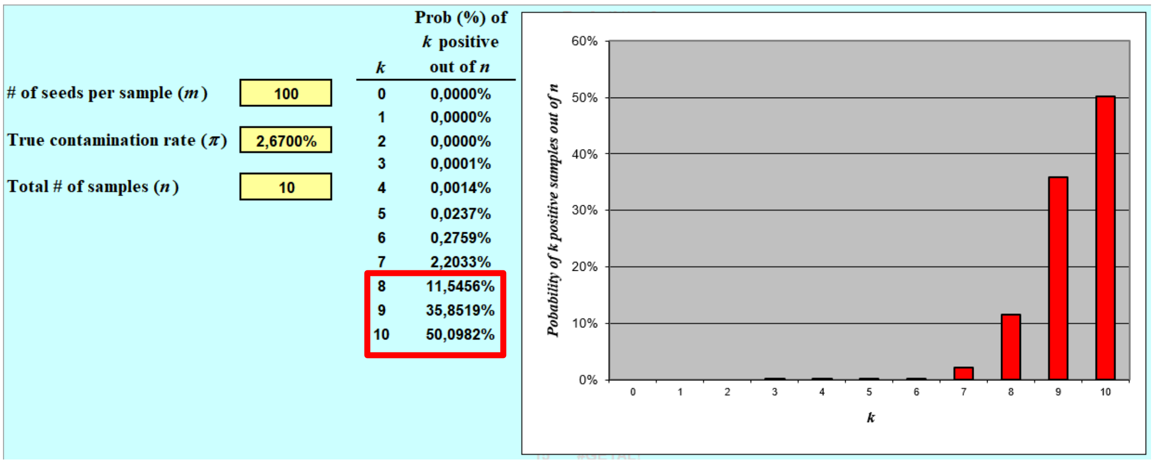
# of Seed Pools	30
# of Seeds per Pool	100
Total Seeds Tested	3000
# Deviants Pools	28

Computed % in sample 2,67 %

Measured property on seed pools

Desired Confidence Level 95 %

Figure 3. Results of the medium infected seed lot using Seedcalc 8.



# of seeds per sample (*m*)	100
True contamination rate (*π*)	2,6700%
Total # of samples (*n*)	10

k	Prob (%) of *k* positive out of *n*
0	0,0000%
1	0,0000%
2	0,0000%
3	0,0001%
4	0,0014%
5	0,0237%
6	0,2759%
7	2,2033%
8	11,5456%
9	35,8519%
10	50,0982%

Figure 4. Expected number of infected samples for the CT medium infected seed lot according to infection rate with the "probability of *k* positive samples out of *n*" tool.

Table 11. CGMMV CT results for all laboratories. A red cell indicates a deviation from expected result. #/# represents the number of positive samples out of a total number of samples tested.

Participant	Healthy	Medium	High
Laboratory 1	0+/8	7+/10	10+/10
Laboratory 2	0+/8	9+/10	10+/10
Laboratory 3	0+/8	10+/10	10+/10
Laboratory 4	0+/8	9+/10	10+/10
Laboratory 5	0+/8	8+/10	10+/10
Laboratory 6	1+/8	10+/10	10+/10
Expected result	**0+/8**	**8 to 10+/10**	**10+/10**
Concordance	**95.8%**	**Not calculated**	**100%**

For the healthy and high infected samples, all participants obtained the expected results with the exception of laboratory 6 that scored one healthy sample as infected. A concordance (reproducibility) of 95.8% and 100% was obtained for the healthy and high infected samples, respectively.

For the medium infected samples, all participants reported results between the expected range of 8 to 10 positive samples, with the exception of laboratory 1 (Table 11).

SqMV & MNSV CT

The CT was performed as a parallel CT for ELISA and RT-qPCR. The samples for the CT were chosen based on homogeneity testing done by RT-qPCR (data not shown). The analysis was defined to compare results between samples, rather than between subsamples, therefore the participants were asked to look at each sample as a whole. The participants received 30 sample bags of 1000 seeds each and were asked to prepare 10 subsamples from each sample (taking into account that cross contamination may occur between subsamples). Analysis at the sample level is reflecting the process done routinely in QC labs in the seed health industry. See raw data in Annex E.4 and E.5 and in Table 12 and 13.

Laboratory 1 was not able to detect the positive controls in both assays, furthermore, the participant declared a high number of inconclusive subsamples. Therefore, laboratory 1 was excluded from the analysis.

Table 12. MNSV CT results per sample. A red cell indicates a deviation from the expected result.

#	Sample	Participant					
		2	3	4	5	6	7
1	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
2	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
3	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
4	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
5	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
6	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
7	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
8	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
9	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
10	MNSV	Positive	Positive	Positive	Positive	Positive	Positive

#	Sample	Participant					
		2	3	4	5	6	7
11	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
12	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
13	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
14	MNSV	Positive	Positive	Positive	Positive	Positive	Positive
15	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
16	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
17	MNSV	Positive	Positive	Positive	Positive	Positive	Positive
18	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
19	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
20	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
21	MNSV	Positive	Positive	Positive	Positive	Positive	Positive
22	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
23	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
24	Healthy	Positive	Negative	Negative	Negative	Negative	Negative
25	Healthy	Negative	Negative	Negative	Positive	Negative	Negative
26	SqMV	Negative	Negative	Negative	Negative	Negative	Negative
27	MNSV	Positive	Positive	Positive	Positive	Positive	Positive
28	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
29	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
30	Healthy	Negative	Negative	Negative	Negative	Negative	Negative

Table 13. SqMV CT results per sample. A red cell indicates a deviation from the expected result.

#	Sample	Participant					
		2	3	4	5	6	7
1	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
2	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
3	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
4	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
5	Healthy	Positive	Negative	Negative	Negative	Negative	Negative
6	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
7	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
8	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
9	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
10	MNSV	Negative	Negative	Negative	Negative	Negative	Negative
11	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
12	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
13	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
14	MNSV	Negative	Negative	Negative	Negative	Negative	Negative
15	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
16	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
17	MNSV	Negative	Negative	Negative	Negative	Negative	Negative
18	Healthy	Negative	Negative	Negative	Negative	Negative	Negative

#	Sample	Participant					
		2	3	4	5	6	7
19	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
20	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
21	MNSV	Negative	Negative	Negative	Negative	Negative	Negative
22	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
23	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
24	Healthy	Positive	Negative	Negative	Negative	Negative	Negative
25	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
26	SqMV	Positive	Positive	Positive	Positive	Positive	Positive
27	MNSV	Negative	Negative	Negative	Negative	Negative	Negative
28	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
29	Healthy	Negative	Negative	Negative	Negative	Negative	Negative
30	Healthy	Negative	Negative	Negative	Negative	Negative	Negative

Table 14. Qualitative MNSV and SqMV ELISA CT results for all laboratories and concordance per infection level. A red cell indicates a deviation from the expected result. #/# represents the number of positive samples out of a total number of samples tested.

Participant	MNSV ELISA		SqMV ELISA	
	Healthy	Infected	Healthy	Infected
Laboratory 2	1+/25	5+/5	2+/23	7+/7
Laboratory 3	0+/25	5+/5	0+/23	7+/7
Laboratory 4	0+/25	5+/5	0+/23	7+/7
Laboratory 5	1+/25	5+/5	0+/23	7+/7
Laboratory 6	0+/25	5+/5	0+/23	7+/7
Laboratory 7	0+/25	5+/5	0+/23	7+/7
Expected result	**0+/25**	**5+/5**	**0+/23**	**7+/7**
Concordance	**97.4%**	**100%**	**97.1%**	**100%**

For the healthy and MNSV infected samples, all participants obtained the expected results with the exception of laboratories 2 and 5, who scored one healthy sample as infected. A concordance (reproducibility) of 97.4% was obtained for the healthy samples and a concordance of 100% for the high infected samples (Table 14). For the healthy and SqMV infected samples all participants obtained the expected results with the exception of laboratory 2, which scored two healthy samples as infected. A concordance (reproducibility) of 97.1% and 100% was obtained for the healthy and infected samples, respectively (Table 14).

Conclusion

For the detection of CGMMV, concordance of 95.8% for the healthy seed lots and 100% for the high infected subsamples revealed that the reproducibility requirements are met for the healthy and high infected seed lots. For the medium infected lots, one participant detected one subsample less than expected. However, when looking at the overall results of the medium infected subsamples, the 53 positive subsamples out of the 60 subsamples tested fall within the expected range. Based on the infection rate of 2.67%, considering each value with a probability higher than 5%, the CT should give between 53 to 59 positives out of the 60 subsamples tested in the CT (see Table 17). Therefore, for the detection of CGMMV, the reproducibility of the ELISA assay is considered fit for purpose.

For the detection of MNSV and SqMV, concordance for all samples, healthy and infected, was above the requirement of 90%. Therefore, also for the detection of MNSV and SqMV, the reproducibility of the ELISA assay is considered fit for purpose.

3.6. Diagnostic performance

Definition ISHI guidelines: *An evaluation of the ability of the method to discriminate between positive and negative seed lots.*

The diagnostic performance requirements will be met when diagnostic sensitivity and specificity are >95%.

Experimental approach

The diagnostic sensitivity and diagnostic specificity, the diagnostic performance parameters of the method in other words, were calculated according to the mathematical formulas in Table 15. In the absence of a reference method, the qualitative data (detected/not detected) generated in the comparative test, along with the expected and obtained results in all participating laboratories, were used in the analysis.

Table 15. Formulas used for diagnostic sensitivity (Dsen) and diagnostic specificity (Dspec) calculation.

	Expected result + (target)	**Expected result – (non-target)**
Obtained result +	True positive (TP)	False positive (FP)
Obtained result -	False negative (FN)	True negative (TN)
	Dsen = TP / (TP + FN) × 100%	**Dspec = TN / (FP + TN) × 100%**

Results

Analysis of the CT results is presented in Table 16 and 17, and the raw data is presented in Annex E. For CGMMV, the medium infected samples are not included in the diagnostic sensitivity and specificity calculations, due to the stability test results.

Analysis of the results for the CGMMV medium infected samples is presented in Table 17. Here the expected number of positive samples according to infection rate is calculated with the “probability of k positive samples out of n” tool (https://www.seedtest.org/en/services-header/tools/seed-health-committee/seed-health-toolbox.html, March 2023). The infection rate comes from the homogeneity and stability test results, see section 3.5. The actual detected samples are compared to the calculated probabilities.

Table 16. Diagnostic sensitivity (Dsen) and diagnostic specificity (Dspec) results for CGMMV, SqMV and MNSV. TP = True positive, FP = False positive, FN = False negative, TN = true negative.

	CGMMV[a]		**SqMV**		**MNSV**	
	Expected result +	**Expected result -**	**Expected result +**	**Expected result -**	**Expected result +**	**Expected result -**
Obtained result +	TP = 60	FP = 1	TP = 42	FP = 2	TP = 30	FP = 2
Obtained result -	FN = 0	TN = 47	FN = 0	TN = 136	FN = 0	TN = 148
Dsen	100%		100%		100%	
Dspec	97.9%		98.6%		98.7%	

[a] Only high infected and healthy samples used for calculations.

Table 17. Analysis of qualitative results for the CGMMV medium infected samples.

# Samples tested	# Positive samples expected[1]	# Positive samples obtained
60	53-59	53

[1] Calculated with the “probability of k positive samples out of n” tool.

Conclusion

For CGMMV (high infected and healthy samples), MNSV and SqMV, the diagnostic sensitivity and the diagnostic specificity were above the 95% threshold. For the CGMMV medium infected samples, the diagnostic sensitivity and specificity could not be calculated, since the stability test, done as part of the CT, showed that the level of infection in the medium infection category decreased with time, but the results observed fell within the expected range of detection.

Therefore, the diagnostic performance is considered to be fit for purpose for the detection of CGMMV, MNSV and SqMV by ELISA.

4. CONCLUSION

The ELISA assay for the detection of CGMMV, MNSV and SqMV has been used routinely by the industry for many years. This validation provides important data to support the use of the assay as a reliable detection tool.

The performance criteria assessed during the validation of the ELISA assay for the detection of CGMMV, MNSV and SqMV in cucurbit seeds have shown that the assay is suitable for detecting infected *Cucurbitaceae* seed lots. The sensitivity experiments showed that the limit of detection (LOD) of the assay is one infected seed out of a subsample of 100 seeds.

Two separate CTs were conducted as part of this validation on healthy and CGMMV/MNSV/SqMV infected seed samples of different *Cucurbitaceae* crops. Six laboratories participated in the CGMMV CT, and seven laboratories participated in a combined CT for MNSV and SqMV. The results of all participants for the detection of CGMMV, MNSV and SqMV in both CTs met the requirements of reproducibility for the healthy and infected seed lots.

In this validation, antisera from Prime Diagnostics were used for the detection of all three viruses. Internal experiments (data not shown) showed that antisera from different manufacturers can be used for the detection of the viruses. As described by ISHI best practices for ELISA assay, it is the responsibility of each laboratory to validate the use of different antisera for the assay before routine use.

5. REFERENCES

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6. ANNEXES

Annex A. ELISA protocol

ISHI protocol for the detection of CGMMV, MNSV and SqMV in Cucurbits Seed by ELISA.

PRE-SCREEN BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Sample size

The recommended minimum sample size is 2,000 seeds with a maximum subsample size of 100 seeds.

Materials

- Coating and conjugated antisera for the target pathogen
- ELISA buffers (Table A.1 to A.5). Note: other buffer compounds can be used according to the antisera supplier recommendations.
- para-nitrophenyl phosphate (pNPP) for substrate solution
- Controls (Table A.6)
- Grinder
- ELISA necessities
- Lab disposables

Table A.1. Coating buffer, pH 9.6.

Compound	Amount/L
Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g

Table A.2. Seed extraction buffer, pH 7.4.

Compound	Amount/L
NaCl	8.0 g
$Na_2HPO_4 \cdot 12H_2O$	14.5 g
KH_2PO_4	1.0 g
Ovalbumin (grade II)	2.0 g
Tween™ 20	10.0 mL
PVP (ELISA grade, mol. wt. 10,000 Da)	20.0 g

Table A.3. Washing buffer PBS/Tween™ 20, pH 7.4.

Compound	Amount/L
NaCl	8.0 g
$Na_2HPO_4 \cdot 12H_2O$	14.5 g
KH_2PO_4	1.0 g
Tween™ 20	1.5 mL

Table A.4. Conjugate buffer, pH 7.4.

Compound	Amount/L
NaCl	8.0 g
$Na_2HPO_4 \cdot 12H_2O$	14.5 g
KH_2PO_4	1.0 g
Tween^{TM} 20	0.5 mL
PVP (ELISA grade, mol. wt. 10,000 Da)	20.0 g
BSA (ELISA grade, e.g. BSA fraction 5)	5.0 g

Table A.5. Substrate buffer, pH 9.6.

Compound	Amount/L
$C_4H_{11}NO_2$	97 mL
HCl (32 %)	15 mL

Table A.6. Types of controls used.

Control type	Description
Positive process control (PPC)	Cucurbit seed with infectious SqMV, CGMMV and MNSV or standardised reference material (flour of seeds or leaves containing SqMV, CGMMV and MNSV)
Negative process control (NPC)	Cucurbit seed free of SqMV, CGMMV and MNSV
Buffer control (BC)	The buffers and reagents used in the ELISA, with no seed/tissue matrix or target pathogen

1. Coating of ELISA plates

1.1. Add an appropriate amount of coating serum specific for the tested virus, according to the manufacturer's instructions, to the coating buffer (Table A.1.) to obtain coating solution.

1.2. Add coating solution, as recommended by the manufacturer, to each well in the ELISA plates.

Note: Using different types of microtiter plates may influence sensitivity.

1.3. Cover the plates with a suitable cover or film.

1.4. Incubate plates for 3 hours at 37 ± 2 °C, or as defined by the supplier.

2. Seed extraction

2.1. Divide each sample into subsamples of a maximum of 100 seeds each. Prepare positive and negative controls (Table A.6). Grind each subsample, the negative process control (NPC) and the positive process control (PPC), to fine flour in a grinder.

Note: Be sure to use a grinder that can be cleaned thoroughly, since cross-contamination is likely during the grinding step.

2.2. From each subsample, weigh out 0.5 g of ground seeds and transfer to a suitable container.

2.3. Add 5 mL of extraction buffer (Table A.2) to each container (ratio of 1:10).

Note: Use the same buffers also for commercial controls.

2.4. Mix/vortex each container thoroughly. Allow extract to settle for at least 5 min on the bench to facilitate pipetting.

2.5. After plate incubation (step 1.4), wash the plates at least three times using washing buffer (Table A.3) to remove residues.

2.6. Immediately after washing of the plates, transfer 100 µL seed extract from each subsample (step 2.4) to two wells to create a duplicate.

2.7. Load 100 µL of each control, in duplicate. Use at least two dilutions, high and low for the positive controls.

2.8. Cover the plates with a suitable lid or film and incubate overnight at 4 ± 2 °C, or as defined by the supplier.

3. Incubation of conjugate

3.1. Add an appropriate dilution of conjugate serum of the tested virus, according to the manufacturer's instructions to the conjugate buffer (Table A.4) to obtain conjugate solution.

3.2. Remove the seed extract from ELISA plates and wash at least three times using washing buffer (Table A.3) to remove residues.

3.3. Immediately after washing the plates, add 100 µL of conjugate solution to each well of the ELISA plates.

3.4. Cover the plates with a suitable cover or film and incubate for 3 hours at 37 ± 2 °C, or as defined by the supplier.

4. Addition of substrate to ELISA plates

4.1. Prepare substrate solution by adding 10 mg para-nitrophenyl phosphate (pNPP) to 20 mL of substrate buffer (Table A.5).

4.2. Remove the conjugate solution from ELISA plates and wash at least three times using washing buffer (Table A.3).

4.3. Add 100 µL of substrate solution to each well.

4.4. Incubate <u>in the dark</u> for 1 hour at room temperature, or as defined by the supplier.

Note: Substrate solution is light sensitive; light influences results and exposure should therefore be avoided as much as possible.

4.5. Measure the extinction value (A_{405}), optical density (OD), with ELISA plate reader.

Note: The source of antiserum is critical. In the comparative test study, the antiserum supplied by Prime Diagnostic was used. If different antisera and buffers are used, or even different lot numbers, it is necessary to verify their performance.

5. Validity of test results

Test results are only valid when all included controls presented in Table A.6 give the expected results, as defined by the internal laboratory validation or by the supplier. It is recommended to use a negative-positive threshold of two-times the background of healthy negative controls.

A subsample is positive when the extinction value (A_{405}), OD, is equal or above the calculated threshold. A subsample is negative when the extinction value (A_{405}), OD, is lower than the calculated threshold.

Annex B. Analytical specificity results.

Table B.1. ELISA inclusivity results. A red cell indicates a positive ELISA result, a green cell indicates a negative ELISA result. Threshold ELISA OD: 2 × average NC (CGMMV = 0.184, SqMV = 0.170, MNSV = 0.180). NA: Not analysed.

Lot	Pathogen	Tissue type	Origin	Crop	Year	ELISA OD 1	ELISA OD 2	Identity confirmation
LBH1	CGMMV	Dry leaf	Israel	Cucumber	2017	3.082	3.099	Grow-out
LBH2	CGMMV	Seed powder	Israel	Watermelon	2017	2.619	2.293	NA
LBH3	CGMMV	Seed powder	Israel	Watermelon	2017	0.233	0.239	NA
LBH4	CGMMV	Seed powder	Israel	Watermelon	2017	1.046	1.112	NA
LBH6	CGMMV	Seeds	Israel	Watermelon	2017	0.831	0.859	NA
LBH102	CGMMV	Seeds	unknown	Melon	2018	2.974	3.236	NA
LBH20	CGMMV	Seeds	Israel	Cucumber	2013	0.646	0.577	NA
LBH17	CGMMV	Seeds	Israel	Melon	2017	0.575	0.572	NA
LBH19	CGMMV	Seeds	Israel	Watermelon	2012	0.437	0.356	NA
LBH21	CGMMV	Seeds	Israel	Melon	2013	0.345	0.321	NA
LBH22	CGMMV	Dry leaf	unknown	Cucumber	2016	3.506	3.028	Grow-out
LBH23	CGMMV	Dry leaf	unknown	Cucumber	2015	3.507	3.575	Grow-out
LBH24	CGMMV	Dry leaf	unknown	Cucumber	2015	4.171	4.080	Grow-out
LBH68	CGMMV	Leaf	Israel	Cucumber	2018	2.321	2.381	PCR+ Sequencing
Or1	CGMMV	Seed powder	India	Watermelon	2015	0.235	0.227	NA
Or2	CGMMV	Seed powder	Israel	Cucumber	Unknown	0.287	0.295	NA
Or3	CGMMV	Seed powder	Israel	Watermelon	2016	1.045	0.935	NA
Or4	CGMMV	Seed powder	Israel	Watermelon	2017	1.076	1.116	NA
Or5	CGMMV	Seed powder	Israel	Cucumber	Unknown	0.205	0.183	NA
Or6	CGMMV	Seed powder	Israel	Melon	2014	0.194	0.215	NA
Or7	CGMMV	Seed powder	Israel	Watermelon	2017	0.227	0.258	NA
Or8	CGMMV	Seed powder	Israel	Watermelon	2017	0.800	0.830	NA
LBH46	CGMMV	Dry leaf	Israel	Watermelon	2010	2.683	2.434	PCR+ Sequencing

Lot	Pathogen	Tissue type	Origin	Crop	Year	ELISA OD 1	ELISA OD 2	Identity confirmation
LBH47	CGMMV	Dry leaf	Israel	Cucumber	2017	2.402	2.662	PCR+ Sequencing
LBH48	CGMMV	Dry leaf	Israel	Cucumber	2011	2.047	1.870	PCR+ Sequencing
LBH49	CGMMV	Dry leaf	Israel	Cucumber	2011	1.996	1.960	PCR+ Sequencing
LBH50	CGMMV	Dry leaf	Israel	Cucumber	2015	3.346	3.123	Grow-out
LBH51	CGMMV	Dry leaf	Israel	Cucumber	2011	2.084	2.362	Grow-out
Or11	CGMMV	Seeds	Israel	Watermelon	2013	0.241	0.294	NA
LBH25	SqMV	Seeds	Unknown	Melon	2018	2.566	2.649	NA
LBH26	SqMV	Dry leaf	Unknown	Pumpkin	2015	3.378	2.834	PCR+ Sequencing
LBH27	SqMV	Dry leaf	Unknown	Pumpkin	2015	1.367	1.221	PCR+ Sequencing
Or17	SqMV	Seeds	Israel	Melon	2015	1.112	1.159	NA
LBH37	SqMV	Seed powder	Unknown	Melon	2015	2.767	2.612	PCR+ Sequencing
LBH38	SqMV	Seed powder	Unknown	Melon	2015	2.679	2.605	NA
LBH61	SqMV	Dry leaf	Israel	Watermelon	2010	2.581	2.625	PCR+ Sequencing
LBH62	SqMV	Dry leaf	Israel	Cucumber	2013	2.569	2.615	PCR+ Sequencing
LBH63	SqMV	Dry leaf	Israel	Cucumber	2012	2.469	2.547	PCR+ Sequencing
ME05	SqMV	Seeds	USA	Melon	2007	3.361	3.156	PCR+ Sequencing
ME15	SqMV	Seeds	France	Melon	2013	3.318	3.346	NA
ME16	SqMV	Seeds	France	Melon	2013	1.859	1.843	NA
LB10	MNSV	Dry leaf	Israel	Melon	2014	3.084	2.830	PCR+ Sequencing
LBH11	MNSV	Dry leaf	Israel	Watermelon	2013	0.217	0.217	Grow-out
LBH12	MNSV	Dry leaf	Unknown	Melon	2017	0.694	0.689	PCR+ Sequencing
LBH13	MNSV	Dry leaf	Unknown	Cucumber	2014	4.627	4.371	PCR+ Sequencing
LBH14	MNSV	Dry leaf	Unknown	Cucumber	2010	4.483	4.371	Grow-out
LBH15	MNSV	Dry leaf	Unknown	Cucumber	2011	4.125	4.142	Grow-out
LBH35	MNSV	Glycerol stock	Unknown	Unknown	Unknown	2.757	2.723	NA
LBH36	MNSV	Dry leaf	Unknown	Unknown	2016	1.560	1.480	Grow-out
LBH39	MNSV	Dry leaf	Israel	Melon	2013	1.281	1.304	PCR+ Sequencing

Lot	Pathogen	Tissue type	Origin	Crop	Year	ELISA OD 1	ELISA OD 2	Identity confirmation
LBH41	MNSV	Dry leaf	Israel	Melon	2017	4.036	3.733	Grow-out
LBH42	MNSV	Dry leaf	Israel	Watermelon	2010	0.232	0.225	PCR+ Sequencing
LBH44	MNSV	Dry leaf	Israel	Watermelon	2004	0.279	0.382	Grow-out
LBH45	MNSV	Dry leaf	Israel	Watermelon	2011	0.246	0.227	Grow-out
LBH28	MNSV	Leaf	Israel	Melon	2018	3.867	3.874	Grow-out
ME31	MNSV	Seeds	France	Melon	2016	0.541	0.521	NA
PC 1	CGMMV	Dry leaf	NA	Commercial PC	NA	3.460	3.704	-
PC 2	SqMV	Dry leaf	NA	Commercial PC	NA	3.122	3.144	-
PC 3	MNSV	Dry leaf	NA	Commercial PC	NA	3.946	3.926	-
NC 1	CGMMV negative	Seeds	NA	Watermelon	NA	0.092	0.093	-
NC 2	SqMV negative	Seeds	NA	Watermelon	NA	0.085	0.085	-
NC 3	MNSV negative	Seeds	NA	Watermelon	NA	0.090	0.089	-

Table B.2. ELISA exclusivity results for CGMMV, MNSV, SqMV. A green cell indicates a negative ELISA result, a red cell indicates a positive ELISA result. Threshold ELISA OD = 2 × average NC (CGMMV = 0.184, SqMV = 0.170, MNSV = 0.180). NA: Not analysed.

Lot	Pathogen	Tissue type	Origin	Crop	Year	CGMMV		SqMV		MNSV	
						OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
LBH30	CVYV	Dry leaf	Israel	Cucumber	2013	0.094	0.098	0.085	0.082	0.089	0.089
LBH31	ZYMV	Dry leaf	Israel	Cucumber	2013	0.104	0.093	0.09	0.086	0.089	0.096
LBH32	WMCSV	Dry leaf	Israel	Watermelon	2015	0.097	0.092	0.084	0.083	0.089	0.087
LBH33	KGMMV	Dry leaf	Unknown	Cucumber	2014	0.112	0.109	0.091	0.092	0.09	0.09
LBH34	ZYMV	Leaf	Israel	Cucumber	2018	0.096	0.096	0.085	0.087	0.091	0.089
LBH52	CFMMV	Dry leaf	Israel	Cucumber	2015	0.103	0.103	0.086	0.088	0.088	0.088
LBH53	CMV	Dry leaf	Israel	Cucumber	2013	0.094	0.099	0.077	0.082	0.088	0.089
LBH54	CMV	Dry leaf	Israel	Cucumber	2006	0.091	0.092	0.085	0.086	0.087	0.087
LBH55	CMV	Dry leaf	Israel	Cucumber	2010	0.090	0.091	0.081	0.083	0.087	0.087
LBH56	CMV	Dry leaf	Israel	Cucumber	2014	0.092	0.093	0.084	0.087	0.088	0.092
LBH57	CMV	Dry leaf	Israel	Cucumber	2016	0.101	0.099	0.090	0.090	0.088	0.093
LBH58	CVYV	Dry leaf	Israel	Cucumber	2010	0.092	0.092	0.088	0.087	0.090	0.092
LBH59	CVYV	Dry leaf	Israel	Cucumber	2011	0.089	0.089	0.088	0.084	0.088	0.085
LBH60	CVYV	Dry leaf	Israel	Cucumber	2016	0.092	0.091	0.083	0.084	0.089	0.098
LBH64	ZYMV	Dry leaf	Israel	Cucumber	2012	0.092	0.092	0.089	0.086	0.096	0.094
LBH65	ZYMV	Dry leaf	Israel	Cucumber	2013	0.094	0.094	0.090	0.088	0.098	0.091
LBH66	ZYMV	Dry leaf	Israel	Cucumber	2018	0.095	0.094	0.086	0.087	0.089	0.09
LBH67	SLCV	Leaf	Israel	Squash	2018	0.115	0.114	0.083	0.086	0.090	0.090
LBH69	WMCSV	Leaf	Israel	Watermelon	2018	0.097	0.098	0.089	0.087	0.091	0.089
LBH46	CGMMV	Dry leaf	Israel	Watermelon	2010	2.683	2.434	0.082	0.084	0.085	0.088
LBH47	CGMMV	Dry leaf	Israel	Cucumber	2017	2.402	2.662	0.082	0.087	0.095	0.107

Lot	Pathogen	Tissue type	Origin	Crop	Year	CGMMV		SqMV		MNSV	
						OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
LBH48	CGMMV	Dry leaf	Israel	Cucumber	2011	2.047	1.870	0.086	0.087	0.094	0.095
LBH49	CGMMV	Dry leaf	Israel	Cucumber	2015	1.996	1.960	0.089	0.096	0.091	0.092
LBH10	MNSV	Dry leaf	Israel	Melon	2014	0.102	0.106	0.090	0.090	3.084	2.830
LBH11	MNSV	Dry leaf	Israel	Watermelon	2013	0.090	0.089	0.084	0.085	0.217	0.217
LBH14	MNSV	Dry leaf	Unknown	Cucumber	2010	0.088	0.089	0.077	0.081	4.483	4.371
LBH25	SqMV	Seeds	Israel	Melon	2007	0.092	0.090	2.566	2.649	0.088	0.092
LBH26	SqMV	Dry leaf	Unknown	Pumpkin	2015	0.095	0.090	3.378	2.834	0.087	0.086
LBH27	SqMV	Dry leaf	Unknown	Pumpkin	2015	0.087	0.088	1.367	1.221	0.093	0.088
Or17	SqMV	Seeds	Israel	Melon	2015	0.091	0.090	1.112	1.159	NA	NA
PC 1	CGMMV	Dry leaf	NA	Commercial PC	NA	3.460	3.704	NA	NA	NA	NA
PC 2	SqMV	Dry leaf	NA	Commercial PC	NA	NA	NA	3.122	3.144	NA	NA
PC 3	MNSV	Dry leaf	NA	Commercial PC	NA	NA	NA	NA	NA	3.946	3.926
NC 1	negative	Seeds	NA	Watermelon	NA	0.092	0.093	NA	NA	NA	NA
NC 2	negative	Seeds	NA	Watermelon	NA	NA	NA	0.085	0.085	NA	NA
NC 3	negative	Seeds	NA	Watermelon	NA	NA	NA	NA	NA	0.090	0.089

Annex C. Analytical sensitivity results.

Table C.1. Analytical sensitivity results for the detection of CGMMV. Threshold ELISA OD = 2 × average NC (0.182). NA: Not analysed.

Lot	Crop	Repetition #	Concentration							
			0.5/100		1/100		2/100		3/100	
			OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
Or31	Watermelon	1	0.211	0.213	0.832	0.857	0.364	0.264	1.082	0.838
		2	0.275	0.262	0.382	0.256	0.563	0.431	0.396	0.56
		3	0.758	0.705	0.252	0.208	0.680	0.554	0.773	0.797
		4	0.474	0.453	0.277	0.291	1.159	0.920	0.746	0.856
Or28	Melon	1	0.220	0.225	0.741	0.725	0.954	0.680	1.174	1.164
		2	0.353	0.315	0.251	0.221	0.898	0.724	0.222	0.237
		3	0.390	0.376	0.569	0.422	1.063	1.007	0.331	0.401
		4	1.448	1.387	0.373	0.287	1.044	1.030	0.544	0.66
LBH100	Cucumber	1	0.782	0.714	0.543	0.602	0.33	0.475	1.469	1.449
		2	0.335	0.320	0.237	0.251	0.475	0.562	0.424	0.315
		3	0.383	0.357	0.203	0.218	0.573	0.644	0.298	0.230
		4	0.377	0.412	0.395	0.556	0.798	0.908	0.414	0.308
Or29	Squash	1	0.141	0.119	0.478	0.429	0.196	0.194	1.108	1.106
		2	0.176	0.125	0.539	0.512	0.22	0.254	0.759	0.750
		3	0.301	0.251	0.545	0.474	0.208	0.219	1.659	1.43
		4	0.342	0.249	0.583	0.590	0.352	0.393	1.602	1.257
LBH78	Pumpkin	1	0.174	0.183	0.336	0.365	0.453	0.405	1.175	1.037
		2	0.247	0.230	0.347	0.366	0.447	0.409	0.447	0.513
		3	0.217	0.254	0.414	0.383	0.715	0.647	0.568	0.620
		4	0.501	0.539	0.499	0.532	0.374	0.373	0.763	0.846
PC CGMMV	Commercial PC		1.165	1.285	NA	NA	NA	NA	NA	NA
NC CGMMV	Melon		0.091	0.091	NA	NA	NA	NA	NA	NA

Table C.2. Analytical sensitivity results for the detection of SqMV. Threshold ELISA OD = 2 × average NC (0.143). NA: Not analysed.

Lot	Crop	Repetition #	Concentration							
			0.5/100		1/100		2/100		3/100	
			OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
Or31	Watermelon	1	0.435	0.543	0.431	0.441	0.573	0.606	0.793	0.843
		2	0.696	0.685	0.343	0.39	0.607	0.596	0.357	0.368
		3	0.147	0.155	0.315	0.179	0.401	0.417	0.478	0.516
		4	0.140	0.138	0.207	0.290	0.501	0.518	0.671	0.700
Or28	Melon	1	0.201	0.198	0.235	0.225	0.536	0.534	0.943	1.907
		2	0.307	0.408	0.104	0.103	1.506	1.531	0.523	0.509
		3	0.142	0.150	0.144	0.143	0.262	0.225	0.637	0.575
		4	0.110	0.123	0.398	0.374	0.616	0.625	0.579	0.469
LBH100	Cucumber	1	0.150	0.150	0.278	0.275	0.207	0.209	0.656	0.657
		2	0.136	0.141	0.141	0.150	1.062	0.566	0.731	0.696
		3	0.215	0.251	0.619	0.567	1.187	1.393	0.601	0.633
		4	0.087	0.098	0.143	0.119	0.432	0.396	0.757	0.595
Or29	Squash	1	0.096	0.091	0.291	0.313	0.783	0.437	0.280	0.307
		2	0.130	0.123	0.327	0.296	0.578	0.610	0.890	1.474
		3	0.316	0.344	0.578	0.494	0.477	0.513	0.711	0.651
		4	0.155	0.148	0.216	0.240	0.212	0.219	1.103	1.369
LBH70	Pumpkin	1	0.090	0.093	0.301	0.283	0.381	0.336	0.925	0.764
		2	0.215	0.172	0.225	0.237	0.776	0.699	0.495	0.467
		3	0.150	0.145	0.408	0.359	0.367	0.357	0.264	0.240
		4	0.103	0.103	0.302	0.319	0.533	0.454	0.466	0.431
PC SqMV	Commercial PC	1	3.129	3.125	NA	NA	NA	NA	NA	NA
NC SqMV	Melon	1	0.072	0.071	NA	NA	NA	NA	NA	NA

Table C.3. Analytical sensitivity results for the detection of MNSV. Threshold ELISA OD = 2 × average NC (0.173). NA: Not analysed.

Lot	Crop	Repetition #	Concentration							
			0.5/100		1/100		2/100		3/100	
			OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
Or31	Watermelon	1	0.210	0.216	0.342	0.354	0.449	0.447	0.733	0.802
		2	0.195	0.204	0.316	0.316	0.438	0.447	0.486	0.510
		3	0.199	0.193	0.296	0.318	0.448	0.489	0.673	0.683
		4	0.217	0.232	0.320	0.300	0.525	0.503	0.685	0.678
Or28	Melon	1	0.235	0.241	0.324	0.294	0.513	0.505	0.682	0.698
		2	0.213	0.205	0.348	0.360	0.518	0.538	0.746	0.762
		3	0.221	0.213	0.317	0.326	0.524	0.509	0.649	0.788
		4	0.229	0.231	0.370	0.312	0.488	0.505	0.638	0.634
LBH100	Cucumber	1	0.228	0.231	0.339	0.404	0.797	0.700	0.929	0.805
		2	0.208	0.209	0.329	0.370	0.634	0.620	0.847	0.755
		3	0.220	0.233	0.329	0.359	0.632	0.604	0.952	0.921
		4	0.248	0.247	0.352	0.369	0.678	0.599	0.880	0.838
Or29	Squash	1	0.254	0.238	0.348	0.313	0.521	0.535	0.692	0.680
		2	0.271	0.245	0.356	0.366	0.552	0.542	0.910	0.779
		3	0.255	0.227	0.419	0.366	0.502	0.469	0.774	0.733
		4	0.245	0.237	0.334	0.330	0.539	0.467	0.640	0.653
LBH70	Pumpkin	1	0.204	0.206	0.367	0.348	0.423	0.466	0.757	0.717
		2	0.199	0.196	0.332	0.325	0.487	0.507	0.776	0.799
		3	0.199	0.205	0.295	0.341	0.494	0.421	0.803	0.750
		4	0.197	0.220	0.292	0.286	0.59	0.651	0.732	0.775
PC MNSV	Commercial PC	1	3.000	3.586	NA	NA	NA	NA	NA	NA
NC MNSV	Melon	1	0.084	0.089	NA	NA	NA	NA	NA	NA

Annex D. Selectivity and repeatability results.

Table D.1. Selectivity and repeatability results for the ELISA detection of CGMMV, MNSV and SqMV. Conc.: Concentration, PC: Positive control, NC: Negative control, NA: Not analysed, Low: spiked with two positive seeds, Medium: spiked with three positive seeds. A red cell indicates a positive result, a green cell indicates a negative result.

Lot	Conc.	CGMMV						SqMV						MNSV					
		Repetition 1		Repetition 2		Repetition 3		Repetition 1		Repetition 2		Repetition 3		Repetition 1		Repetition 2		Repetition 3	
OR20	Low	0.329	0.337	0.341	0.349	0.291	0.274	1.297	1.303	1.030	1.080	1.261	1.322	0.221	0.216	0.287	0.250	0.256	0.255
	Medium	0.369	0.405	0.417	0.434	0.347	0.353	1.680	1.612	1.348	0.987	0.594	0.858	0.720	0.710	0.746	0.726	0.796	0.808
OR22	Low	0.607	0.679	0.672	0.648	0.623	0.614	1.581	1.496	1.343	1.208	0.513	1.146	0.288	0.274	0.312	0.316	0.290	0.281
	Medium	1.065	1.176	1.181	1.189	1.151	1.092	1.761	1.685	1.347	1.355	1.069	1.488	0.979	1.029	0.934	1.001	1.048	0.986
OR26	Low	0.394	0.466	0.440	0.439	0.380	0.374	1.077	0.883	1.176	0.886	0.733	0.436	0.360	0.342	0.370	3.373	0.404	0.399
	Medium	0.453	0.458	0.383	0.387	0.381	0.376	0.091	0.091	0.093	0.092	0.094	0.097	1.261	1.243	1.440	1.384	1.484	1.445
OR28	Low	0.353	0.346	0.325	0.343	0.296	0.282	1.959	1.766	2.216	1.973	0.802	0.729	0.301	0.304	0.298	0.304	0.303	0.316
	Medium	0.467	0.463	0.645	0.533	0.384	0.381	2.301	2.166	1.916	2.022	1.969	2.027	1.008	1.004	1.091	1.008	1.195	1.178
OR29	Low	0.354	0.351	0.309	0.325	0.258	0.249	0.277	0.265	0.292	0.262	0.248	0.233	0.314	0.327	0.321	0.340	0.314	0.327
	Medium	0.358	0.356	0.321	0.379	0.314	0.309	0.356	0.326	0.265	0.254	0.232	0.235	0.939	0.904	0.922	0.947	1.067	1.057
OR31	Low	0.500	0.484	0.446	0.541	0.405	0.399	1.094	1.188	0.960	0.850	0.813	0.859	0.312	0.315	0.359	0.356	0.328	0.366
	Medium	0.397	0.404	0.325	0.337	0.349	0.347	1.369	1.396	1.115	1.059	1.145	1.411	1.098	1.107	1.236	1.213	1.186	1.303
LBH70	Low	0.486	0.455	0.298	0.303	0.356	0.340	1.155	1.152	1.261	1.021	0.675	0.586	0.266	0.271	0.221	0.225	0.266	0.271
	Medium	0.503	0.514	0.376	0.517	0.397	0.394	1.322	1.287	1.315	1.547	0.883	0.694	1.018	1.017	0.969	0.996	1.106	1.086
LBH71	Low	0.556	0.551	0.528	0.493	0.441	0.426	1.392	1.427	1.282	1.236	1.534	2.125	0.353	0.365	0.376	0.412	0.374	0.343
	Medium	0.323	0.324	0.296	0.299	0.267	0.261	1.211	1.140	0.888	0.847	0.839	1.267	1.366	1.486	1.426	1.309	1.433	1.436
LBH77	Low	0.446	0.430	0.379	0.343	0.337	0.324	1.223	1.312	0.952	0.951	0.990	1.236	0.211	0.227	0.240	0.292	0.211	0.227
	Medium	0.679	0.660	0.695	0.545	0.557	0.544	1.412	1.424	1.070	1.052	1.671	1.738	0.784	0.644	0.883	0.887	0.665	0.994
LBH78	Low	0.436	0.442	0.355	0.324	0.557	0.535	0.995	0.997	0.937	0.985	0.794	1.231	0.288	0.288	0.317	0.324	0.288	0.288
	Medium	0.708	0.676	0.494	0.492	0.554	0.547	2.160	2.126	1.677	1.827	1.129	1.882	0.914	0.896	0.959	0.952	1.076	1.079
LBH79	Low	0.338	0.314	0.248	0.238	0.262	0.249	0.783	0.944	1.036	0.876	0.638	0.816	0.277	0.276	0.287	0.286	0.298	0.294

Lot	Conc.	CGMMV						SqMV						MNSV					
		Repetition 1		Repetition 2		Repetition 3		Repetition 1		Repetition 2		Repetition 3		Repetition 1		Repetition 2		Repetition 3	
	Medium	0.409	0.398	0.409	0.558	0.317	0.316	1.471	1.551	1.314	1.378	1.594	1.866	0.987	0.953	1.021	0.951	1.124	1.106
LBH81	Low	0.554	0.564	0.508	0.390	0.418	0.412	1.376	1.351	1.029	1.226	1.513	1.788	0.217	0.212	0.222	0.227	0.183	0.193
	Medium	0.686	0.672	0.524	0.425	0.543	0.525	1.721	1.689	1.273	1.248	2.065	2.081	0.839	0.863	0.956	0.972	0.975	0.957
LBH83	Low	0.510	0.489	0.381	0.326	0.392	0.373	1.661	1.651	0.674	0.729	0.915	1.017	0.250	0.242	0.238	0.252	0.205	0.236
	Medium	0.791	0.790	0.549	0.500	0.682	0.665	1.472	1.396	1.094	1.129	1.290	1.505	0.905	0.857	0.928	0.903	1.030	0.077
LBH85	Low	0.435	0.425	0.285	0.262	0.363	0.362	1.014	1.035	0.966	0.865	0.920	1.056	0.268	0.271	0.274	0.265	0.238	0.240
	Medium	0.614	0.603	0.373	0.367	0.584	0.505	1.113	1.164	1.204	1.169	1.387	1.274	1.061	0.992	1.024	1.021	1.094	1.090
LBH92	Low	0.606	0.602	0.463	0.433	0.512	0.510	1.495	1.523	1.520	1.573	1.671	1.646	0.295	0.310	0.324	0.337	0.295	0.310
	Medium	0.672	0.707	0.507	0.502	0.531	0.557	2.087	2.128	1.858	1.833	2.360	2.875	1.111	1.077	0.677	1.087	1.207	1.233
LBH94	Low	0.347	0.339	0.248	0.232	0.271	0.271	0.675	0.777	0.519	0.557	1.159	1.032	0.263	0.263	0.258	0.254	0.274	0.289
	Medium	0.410	0.417	0.301	0.293	0.317	0.324	1.086	1.112	0.899	0.928	1.285	1.636	0.978	0.942	1.007	0.987	1.118	1.137
LBH95	Low	0.271	0.309	0.190	0.300	0.239	0.235	0.545	0.692	0.518	0.531	0.511	0.527	0.152	0.156	0.163	0.166	0.144	0.153
	Medium	0.367	0.365	0.227	0.254	0.288	0.299	0.667	0.650	0.693	0.787	0.548	0.760	0.474	0.492	0.574	0.558	0.531	0.506
LBH98	Low	0.308	0.309	0.203	0.215	0.283	0.285	0.596	0.582	0.612	0.557	0.555	0.572	0.345	0.349	0.345	0.355	0.371	0.354
	Medium	0.262	0.286	0.182	0.192	0.263	0.250	0.944	0.826	1.049	1.083	0.859	0.967	1.021	1.108	0.974	1.004	1.143	1.073
LBH99	Low	0.200	0.208	0.248	0.250	0.294	0.299	0.919	0.913	0.921	0.926	0.965	1.197	0.277	0.270	0.283	0.257	0.264	0.269
	Medium	0.162	0.190	0.208	0.204	0.246	0.253	0.969	0.983	0.961	1.006	1.447	1.495	1.049	0.992	0.965	0.918	1.075	1.038
LBH101	Low	0.149	0.168	0.184	0.178	0.206	0.210	0.643	0.576	0.500	0.487	0.670	0.926	0.286	0.292	0.341	0.299	0.304	0.305
	Medium	0.185	0.185	0.232	0.218	0.275	0.278	0.725	0.733	0.595	0.655	0.816	1.016	1.002	1.029	1.053	1.016	1.085	1.078
PC CGMMV	-	1.733	1.852	2.49	2.225	1.946	1.909	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PC SqMV	-	NA	NA	NA	NA	NA	NA	3.028	3.283	3.112	3.169	3.675	4.109	NA	NA	NA	NA	NA	NA
PC MNSV	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.514	4.500	3.514	3.880	3.672	3.478
NC CGMMV	-	0.084	0.084	0.076	0.079	0.084	0.084	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Lot	Conc.	CGMMV						SqMV						MNSV					
		Repetition 1		Repetition 2		Repetition 3		Repetition 1		Repetition 2		Repetition 3		Repetition 1		Repetition 2		Repetition 3	
NC SqMV	-	NA	NA	NA	NA	NA	NA	0.091	0.092	0.093	0.091	0.095	0.096	NA	NA	NA	NA	NA	NA
NC MNSV	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.095	0.094	0.093	0.095	0.095	0.093

Annex E. Reproducibility results.

Annex E.1. CT plan for CGMMV.

2021_June_Compar
ative Test plan_Cucu

Annex E.2. CT plan for MNSV and SqMV.

2019_May_Comapra
tive Test Plan Cucur

Table E.3. Reproducibility results for the ELISA detection of CGMMV. A red cell indicates a positive result. A green cell indicates a negative result. Threshold ELISA OD = 2 × average NC.

Sample#	Level	Crop	Laboratory 1		Laboratory 2		Laboratory 3		Laboratory 4		Laboratory 5		Laboratory 6		Stability test	
			OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
5	Healthy	Cucumber	0.093	0.095	0.092	0.099	0.157	0.157	0.119	0.089	0.058	0.058	0.097	0.084	0.1	0.1
18	Healthy	Cucumber	0.100	0.107	0.095	0.098	0.176	0.170	0.087	0.081	0.057	0.055	0.082	0.083	0.099	0.1
10	Healthy	Melon	0.094	0.093	0.108	0.096	0.173	0.166	0.093	0.092	0.063	0.054	0.086	0.083	0.104	0.11
28	Healthy	Melon	0.09	0.089	0.095	0.097	0.165	0.171	0.082	0.081	0.057	0.057	0.092	0.088	0.092	0.094
1	Healthy	Squash	0.100	0.094	0.106	0.096	0.143	0.151	0.081	0.084	0.058	0.055	0.099	0.091	0.102	0.102
23	Healthy	Squash	0.092	0.096	0.100	0.099	0.155	0.143	0.086	0.090	0.052	0.053	0.085	0.087	0.097	0.102
2	Healthy	Watermelon	0.083	0.087	0.140	0.143	0.224	0.199	0.101	0.102	0.073	0.066	0.124	0.117	0.124	0.125
14	Healthy	Watermelon	0.097	0.105	0.121	0.122	0.203	0.189	0.096	0.096	0.070	0.066	0.254	0.190	0.088	0.123
4	Medium	Cucumber	0.233	0.226	0.331	0.322	2.043	1.999	1.194	1.152	0.499	0.534	1.196	1.170	0.387	0.418
11	Medium	Cucumber	0.266	0.265	0.874	0.856	2.317	2.122	1.107	1.151	0.106	0.109	1.312	1.332	1.846	1.779
19	Medium	Cucumber	1.034	1.025	1.673	1.704	2.220	2.070	0.247	0.242	0.402	0.389	0.303	0.306	0.929	0.863
24	Medium	Cucumber	4.180	4.010	0.784	0.797	1.317	1.230	1.314	1.276	0.529	0.586	0.447	0.456	1.705	1.689
27	Medium	Cucumber	2.496	2.283	1.516	1.628	1.528	1.504	0.981	0.958	0.509	0.551	1.425	1.280	1.842	1.835
7	Medium	Squash	0.092	0.091	0.234	0.234	0.259	0.262	0.319	0.347	0.210	0.250	0.260	0.271	0.157	0.156
8	Medium	Squash	0.097	0.094	0.319	0.307	0.398	0.370	0.165	0.131	0.132	0.144	1.116	1.155	0.705	0.686
13	Medium	Squash	0.096	0.100	0.141	0.151	0.451	0.450	0.103	0.105	0.143	0.126	1.422	1.423	0.594	0.761
17	Medium	Squash	0.435	0.493	0.244	0.268	0.530	0.470	0.196	0.191	0.161	0.160	0.832	0.814	0.481	0.440
21	Medium	Squash	0.245	0.241	0.215	0.219	0.423	0.404	0.467	0.526	0.101	0.117	0.874	0.978	0.123	0.134
6	High	Melon	1.287	1.312	0.435	0.455	0.832	0.883	0.301	0.318	0.209	0.204	1.224	1.167	0.632	0.630
9	High	Melon	2.861	2.861	0.336	0.314	0.812	0.912	0.347	0.355	0.324	0.236	0.876	0.836	0.537	0.554
15	High	Melon	0.985	1.038	0.320	0.355	0.706	0.559	0.314	0.297	0.243	0.239	1.157	1.174	0.623	0.609
25	High	Melon	2.437	2.137	0.293	0.282	0.628	0.580	0.381	0.391	0.412	0.426	0.942	0.913	0.969	1.066
26	High	Melon	1.216	1.188	0.542	0.853	0.614	0.619	0.475	0.463	0.246	0.229	0.732	0.802	0.376	0.403
3	High	Watermelon	3.648	3.358	2.530	2.201	2.232	2.123	1.099	1.063	0.398	0.425	1.532	1.450	1.896	1.869

Sample#	Level	Crop	Laboratory 1		Laboratory 2		Laboratory 3		Laboratory 4		Laboratory 5		Laboratory 6		Stability test	
			OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
12	High	Watermelon	3.816	3.664	2.233	2.188	2.316	2.296	1.179	1.213	0.699	0.502	1.163	1.201	1.587	1.482
16	High	Watermelon	3.486	3.465	1.875	2.005	2.361	2.241	0.918	0.993	0.467	0.528	1.483	1.504	1.864	1.859
20	High	Watermelon	3.817	3.688	2.123	2.079	2.418	2.287	1.076	1.084	0.592	0.403	1.419	1.474	1.802	1.805
22	High	Watermelon	3.365	3.201	2.075	1.991	2.381	2.294	1.054	1.004	0.489	0.495	1.297	1.394	1.970	1.864

Table E.4. Reproducibility results for the ELISA detection of SqMV. A red cell indicates a positive result. A green cell indicates a negative result.

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
1	Healthy	1	0.042	0.041	0.072	0.038	0.043	0.113	0.112	0.062	0.061	0.110	0.108
		2	0.041	0.037	0.078	0.039	0.045	0.105	0.106	0.061	0.061	0.109	0.106
		3	0.040	0.040	0.075	0.041	0.045	0.107	0.107	0.059	0.060	0.110	0.096
		4	0.039	0.035	0.079	0.041	0.044	0.103	0.102	0.061	0.062	0.118	0.098
		5	0.040	0.036	0.077	0.042	0.050	0.103	0.103	0.059	0.060	0.110	0.111
		6	0.039	0.036	0.076	0.040	0.046	0.109	0.104	0.061	0.060	0.108	0.107
		7	0.038	0.035	0.074	0.038	0.045	0.105	0.109	0.059	0.060	0.107	0.109
		8	0.038	0.031	0.078	0.039	0.043	0.107	0.106	0.060	0.061	0.095	0.094
		9	0.038	0.033	0.074	0.043	0.041	0.109	0.106	0.060	0.073	0.110	0.109
		10	0.038	0.037	0.076	0.047	0.048	0.107	0.107	0.067	0.092	0.107	0.106
2	Healthy	1	0.042	0.039	0.070	0.043	0.046	0.106	0.108	0.058	0.063	0.108	0.109
		2	0.044	0.040	0.083	0.044	0.046	0.103	0.103	0.064	0.066	0.106	0.096
		3	0.040	0.032	0.077	0.047	0.047	0.106	0.107	0.067	0.074	0.115	0.117
		4	0.039	0.035	0.078	0.043	0.041	0.100	0.101	0.063	0.065	0.112	0.108
		5	0.040	0.029	0.081	0.046	0.048	0.103	0.103	0.063	0.060	0.111	0.110
		6	0.039	0.035	0.083	0.042	0.045	0.102	0.103	0.064	0.068	0.109	0.098
		7	0.038	0.035	0.078	0.046	0.046	0.102	0.103	0.094	0.069	0.114	0.112
		8	0.040	0.036	0.079	0.049	0.050	0.106	0.106	0.060	0.061	0.108	0.100
		9	0.038	0.032	0.077	0.045	0.047	0.103	0.103	0.059	0.069	0.109	0.107
		10	0.039	0.032	0.081	0.048	0.047	0.106	0.105	0.067	0.070	0.109	0.120
3	Healthy	1	0.042	0.039	0.069	0.045	0.045	0.105	0.106	0.054	0.055	0.108	0.108
		2	0.043	0.034	0.072	0.042	0.041	0.101	0.102	0.058	0.057	0.103	0.105
		3	0.042	0.039	0.070	0.045	0.042	0.108	0.107	0.055	0.056	0.106	0.104
		4	0.040	0.040	0.073	0.042	0.041	0.105	0.104	0.056	0.056	0.107	0.104

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.043	0.040	0.072	0.039	0.039	0.103	0.104	0.057	0.057	0.103	0.106
		6	0.041	0.035	0.074	0.042	0.041	0.113	0.114	0.058	0.060	0.104	0.104
		7	0.041	0.039	0.070	0.039	0.039	0.107	0.107	0.059	0.058	0.104	0.093
		8	0.039	0.039	0.073	0.040	0.043	0.109	0.110	0.057	0.060	0.093	0.104
		9	0.039	0.037	0.071	0.042	0.043	0.106	0.107	0.056	0.064	0.110	0.107
		10	0.039	0.031	0.075	0.040	0.040	0.103	0.104	0.055	0.056	0.097	0.110
4	Healthy	1	0.041	0.039	0.072	0.042	0.040	0.104	0.104	0.057	0.057	0.097	0.104
		2	0.042	0.034	0.073	0.040	0.040	0.096	0.096	0.056	0.058	0.108	0.093
		3	0.042	0.040	0.071	0.052	0.050	0.100	0.102	0.055	0.057	0.106	0.107
		4	0.042	0.040	0.075	0.044	0.043	0.100	0.100	0.056	0.059	0.104	0.102
		5	0.042	0.040	0.071	0.041	0.040	0.098	0.098	0.057	0.056	0.105	0.104
		6	0.040	0.032	0.071	0.042	0.043	0.096	0.096	0.057	0.059	0.130	0.108
		7	0.043	0.022	0.072	0.043	0.045	0.098	0.098	0.057	0.057	0.096	0.107
		8	0.039	0.027	0.074	0.040	0.041	0.096	0.096	0.054	0.057	0.106	0.103
		9	0.038	0.028	0.073	0.041	0.043	0.101	0.101	0.055	0.059	0.105	0.104
		10	0.039	0.033	0.072	0.042	0.040	0.095	0.095	0.057	0.059	0.097	0.105
5	Healthy	1	0.167	0.157	0.079	0.032	0.037	0.106	0.107	0.073	0.068	0.102	0.102
		2	0.148	0.114	0.077	0.034	0.039	0.101	0.102	0.059	0.072	0.109	0.096
		3	0.202	0.168	0.081	0.032	0.036	0.107	0.108	0.059	0.057	0.106	0.104
		4	0.220	0.193	0.078	0.035	0.039	0.108	0.109	0.061	0.058	0.105	0.099
		5	0.248	0.186	0.077	0.035	0.040	0.106	0.106	0.057	0.072	0.105	0.103
		6	0.112	0.111	0.077	0.030	0.034	0.105	0.105	0.064	0.064	0.104	0.096
		7	0.245	0.115	0.072	0.034	0.033	0.108	0.108	0.059	0.060	0.104	0.107
		8	0.146	0.133	0.073	0.030	0.039	0.107	0.107	0.060	0.062	0.101	0.096
		9	0.118	0.115	0.076	0.041	0.037	0.106	0.106	0.058	0.060	0.097	0.101

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.082	0.069	0.076	0.037	0.039	0.106	0.107	0.058	0.059	0.099	0.097
6	Healthy	1	0.042	0.040	0.086	0.038	0.039	0.106	0.106	0.056	0.057	0.102	0.095
		2	0.039	0.034	0.082	0.041	0.041	0.097	0.096	0.059	0.061	0.103	0.095
		3	0.040	0.034	0.088	0.039	0.041	0.100	0.100	0.060	0.060	0.104	0.104
		4	0.040	0.038	0.098	0.032	0.038	0.105	0.106	0.058	0.059	0.107	0.098
		5	0.041	0.042	0.091	0.039	0.041	0.097	0.097	0.048	0.063	0.105	0.106
		6	0.038	0.033	0.081	0.034	0.039	0.099	0.100	0.061	0.061	0.098	0.104
		7	0.037	0.032	0.091	0.042	0.041	0.100	0.100	0.061	0.058	0.103	0.106
		8	0.036	0.036	0.088	0.045	0.044	0.102	0.103	0.058	0.058	0.110	0.101
		9	0.036	0.033	0.096	0.042	0.040	0.098	0.098	0.059	0.061	0.107	0.106
		10	0.036	0.033	0.080	0.044	0.044	0.097	0.097	0.059	0.061	0.105	0.104
7	Healthy	1	0.043	0.043	0.076	0.043	0.040	0.102	0.103	0.057	0.057	0.098	0.104
		2	0.043	0.035	0.079	0.041	0.041	0.105	0.107	0.057	0.058	0.093	0.114
		3	0.041	0.034	0.075	0.042	0.042	0.109	0.109	0.055	0.056	0.094	0.103
		4	0.043	0.035	0.075	0.035	0.033	0.110	0.110	0.054	0.054	0.103	0.093
		5	0.043	0.040	0.075	0.037	0.037	0.104	0.104	0.055	0.058	0.095	0.104
		6	0.041	0.036	0.078	0.043	0.043	0.105	0.107	0.059	0.053	0.102	0.105
		7	0.040	0.031	0.077	0.038	0.041	0.110	0.108	0.056	0.054	0.105	0.105
		8	0.040	0.039	0.079	0.041	0.042	0.103	0.104	0.055	0.056	0.105	0.104
		9	0.040	0.036	0.076	0.040	0.042	0.099	0.100	0.053	0.058	0.103	0.092
		10	0.041	0.035	0.077	0.036	0.041	0.099	0.102	0.068	0.059	0.106	0.106
8	SqMV contaminated	1	1.550	1.392	1.820	0.299	0.306	0.150	0.153	0.061	0.061	0.097	0.107
		2	0.064	0.072	2.388	0.264	0.286	0.902	0.966	0.706	0.842	0.107	0.107
		3	0.045	0.043	2.823	2.034	1.927	0.837	0.881	0.701	0.721	0.110	0.109
		4	0.043	0.043	0.820	0.187	0.189	1.257	1.351	0.614	0.646	0.340	0.587

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	1.758	1.815	2.747	0.043	0.042	0.594	0.637	0.115	0.113	0.107	0.107
		6	2.223	2.092	1.026	0.046	0.046	0.179	0.184	0.237	0.211	0.107	0.107
		7	0.108	0.104	0.265	0.044	0.042	0.132	0.131	0.060	0.059	0.106	0.094
		8	0.040	0.040	2.705	2.241	2.201	0.126	0.126	0.060	0.059	0.106	0.109
		9	0.047	0.042	0.821	0.366	0.368	0.348	0.371	0.059	0.060	0.113	0.355
		10	1.931	1.879	2.273	1.157	1.092	1.081	1.169	0.060	0.060	0.170	0.185
9	SqMV contaminated	1	0.041	0.042	2.537	0.039	0.042	1.287	1.416	0.060	0.063	0.101	0.108
		2	1.738	1.838	0.868	0.402	0.419	0.161	0.160	0.645	0.756	0.104	0.109
		3	0.142	0.154	0.500	0.088	0.086	0.233	0.249	0.704	0.692	0.098	0.109
		4	0.049	0.050	0.152	0.891	0.985	0.837	0.944	0.062	0.060	0.097	0.115
		5	0.044	0.043	0.131	0.113	0.114	0.850	0.957	0.059	0.059	0.318	0.335
		6	0.554	0.584	2.926	0.107	0.115	0.251	0.273	1.257	1.171	0.288	0.252
		7	1.500	1.612	2.888	0.516	0.516	1.056	1.188	0.416	0.439	0.477	0.620
		8	2.462	2.572	2.850	1.748	1.967	1.392	1.542	0.881	0.880	0.415	0.523
		9	2.004	2.069	2.882	0.940	0.965	0.116	0.116	0.058	0.059	0.284	0.328
		10	0.109	0.108	1.369	0.413	0.407	0.118	0.120	0.058	0.060	0.111	0.135
10	MNSV contaminated	1	0.039	0.044	0.068	0.036	0.037	0.108	0.108	0.060	0.060	0.106	0.108
		2	0.039	0.039	0.073	0.040	0.038	0.102	0.101	0.057	0.060	0.104	0.104
		3	0.040	0.037	0.068	0.039	0.039	0.108	0.105	0.057	0.061	0.092	0.104
		4	0.038	0.039	0.064	0.035	0.037	0.097	0.097	0.059	0.060	0.094	0.105
		5	0.039	0.037	0.068	0.040	0.040	0.098	0.095	0.056	0.059	0.112	0.104
		6	0.039	0.036	0.071	0.042	0.039	0.108	0.110	0.060	0.058	0.094	0.107
		7	0.037	0.036	0.068	0.039	0.038	0.099	0.098	0.062	0.061	0.104	0.106
		8	0.037	0.035	0.070	0.044	0.041	0.099	0.098	0.059	0.058	0.099	0.095
		9	0.037	0.035	0.068	0.039	0.042	0.103	0.103	0.058	0.058	0.097	0.127

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.037	0.035	0.070	0.042	0.041	0.100	0.101	0.061	0.062	0.103	0.103
11	SqMV contaminated	1	1.957	2.352	0.072	0.686	0.592	0.121	0.123	0.062	0.064	0.095	0.099
		2	1.197	1.478	0.088	0.211	0.217	0.173	0.181	0.142	0.185	0.104	0.108
		3	0.164	0.204	2.624	2.268	2.014	0.320	0.339	0.070	0.068	0.111	0.106
		4	0.045	0.043	1.793	0.501	0.518	0.919	1.022	0.832	0.856	0.107	0.107
		5	0.042	0.040	1.129	0.231	0.216	0.163	0.161	0.062	0.075	0.110	0.105
		6	1.229	1.280	0.585	1.297	1.313	0.553	0.604	1.077	0.994	0.334	0.317
		7	0.122	0.121	1.886	1.538	1.663	0.123	0.123	0.796	0.841	0.109	0.106
		8	0.041	0.041	2.760	0.461	0.471	0.121	0.118	0.939	0.699	0.104	0.104
		9	0.131	0.139	2.249	3.452	3.483	0.124	0.125	0.440	0.556	0.092	0.101
		10	0.051	0.049	2.238	2.557	2.844	0.112	0.111	0.674	0.618	0.104	0.104
12	Healthy	1	0.042	0.041	0.071	0.041	0.040	0.099	0.101	0.056	0.052	0.107	0.108
		2	0.041	0.039	0.075	0.038	0.040	0.097	0.098	0.054	0.062	0.104	0.105
		3	0.041	0.039	0.071	0.037	0.032	0.100	0.100	0.058	0.065	0.105	0.106
		4	0.042	0.040	0.072	0.04	0.041	0.100	0.101	0.059	0.053	0.092	0.098
		5	0.042	0.040	0.070	0.036	0.036	0.101	0.101	0.053	0.058	0.103	0.105
		6	0.040	0.038	0.073	0.047	0.040	0.097	0.097	0.061	0.057	0.102	0.102
		7	0.038	0.038	0.070	0.041	0.039	0.101	0.101	0.064	0.056	0.093	0.104
		8	0.038	0.037	0.073	0.048	0.037	0.101	0.100	0.054	0.056	0.105	0.104
		9	0.038	0.037	0.071	0.045	0.042	0.097	0.097	0.056	0.057	0.107	0.107
		10	0.039	0.041	0.074	0.036	0.039	0.102	0.101	0.057	0.061	0.105	0.105
13	SqMV contaminated	1	1.381	1.709	0.845	3.471	3.409	0.104	0.105	0.062	0.070	0.104	0.099
		2	0.056	0.058	2.660	0.346	0.378	0.110	0.109	0.063	0.071	0.099	0.104
		3	2.658	3.151	0.516	1.591	1.686	0.549	0.558	1.027	1.028	0.107	0.098
		4	3.071	3.217	0.596	1.261	1.323	0.152	0.149	0.703	0.747	0.109	0.097

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.142	0.161	0.384	1.102	1.093	0.770	0.783	0.065	0.064	0.103	0.097
		6	0.046	0.047	0.195	1.219	1.387	1.179	1.168	0.064	0.065	0.105	0.104
		7	0.045	0.045	0.139	0.522	0.487	0.420	0.433	1.328	1.451	0.104	0.103
		8	0.039	0.039	0.124	0.379	0.376	0.648	0.670	0.061	0.063	0.416	0.364
		9	0.446	0.517	0.194	3.229	3.282	0.190	0.193	0.060	0.065	0.103	0.109
		10	0.043	0.046	0.081	1.443	1.747	0.651	0.717	0.860	0.869	0.365	0.366
14	MNSV contaminated	1	0.040	0.042	0.072	0.035	0.039	0.110	0.112	0.057	0.059	0.102	0.106
		2	0.039	0.039	0.075	0.036	0.039	0.097	0.097	0.062	0.062	0.107	0.111
		3	0.036	0.038	0.069	0.039	0.040	0.098	0.098	0.062	0.065	0.093	0.105
		4	0.039	0.037	0.072	0.035	0.040	0.099	0.101	0.067	0.059	0.104	0.104
		5	0.039	0.036	0.070	0.041	0.044	0.097	0.098	0.058	0.055	0.104	0.103
		6	0.036	0.035	0.074	0.035	0.037	0.096	0.097	0.061	0.061	0.095	0.096
		7	0.036	0.035	0.067	0.035	0.037	0.099	0.100	0.065	0.063	0.091	0.104
		8	0.036	0.035	0.072	0.050	0.044	0.101	0.102	0.059	0.056	0.107	0.101
		9	0.036	0.035	0.069	0.040	0.041	0.098	0.098	0.058	0.064	0.093	0.104
		10	0.037	0.035	0.075	0.041	0.043	0.105	0.105	0.062	0.064	0.096	0.111
15	SqMV contaminated	1	0.044	0.046	2.633	1.169	1.168	0.115	0.118	0.059	0.058	1.089	0.622
		2	0.040	0.042	1.018	0.047	0.043	0.710	0.766	0.054	0.060	0.106	0.105
		3	0.043	0.042	1.117	0.082	0.075	0.842	0.875	0.062	0.063	0.106	0.104
		4	0.044	0.046	1.744	0.154	0.165	0.173	0.179	0.889	0.866	0.103	0.108
		5	0.975	0.970	2.948	2.194	2.243	0.804	0.866	0.080	0.100	0.445	0.432
		6	0.128	0.126	3.323	3.055	3.094	0.343	0.367	0.063	0.066	0.772	0.793
		7	1.787	1.713	2.140	0.048	0.053	0.595	0.636	0.070	0.065	0.108	0.108
		8	0.082	0.077	1.969	0.366	0.376	0.236	0.251	0.060	0.060	0.589	0.397
		9	0.044	0.042	1.225	0.051	0.043	0.997	1.088	0.618	0.556	0.110	0.109

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	2.937	2.860	0.547	0.136	0.137	1.013	1.136	0.773	0.880	0.186	0.193
16	Healthy	1	0.044	0.043	0.075	0.039	0.04	0.103	0.104	0.055	0.058	0.105	0.098
		2	0.044	0.043	0.078	0.035	0.037	0.096	0.097	0.055	0.055	0.105	0.106
		3	0.042	0.042	0.071	0.033	0.033	0.104	0.103	0.061	0.056	0.093	0.104
		4	0.043	0.042	0.077	0.042	0.040	0.099	0.102	0.054	0.054	0.102	0.102
		5	0.042	0.042	0.073	0.038	0.038	0.099	0.101	0.057	0.060	0.094	0.110
		6	0.042	0.040	0.079	0.038	0.040	0.096	0.097	0.059	0.074	0.103	0.107
		7	0.040	0.041	0.075	0.040	0.038	0.101	0.102	0.058	0.055	0.106	0.108
		8	0.039	0.038	0.075	0.039	0.028	0.096	0.097	0.056	0.057	0.105	0.104
		9	0.038	0.040	0.073	0.041	0.042	0.101	0.102	0.056	0.060	0.104	0.095
		10	0.040	0.039	0.081	0.033	0.034	0.100	0.101	0.060	0.058	0.106	0.106
17	MNSV contaminated	1	0.039	0.038	0.068	0.034	0.038	0.104	0.105	0.058	0.059	0.104	0.093
		2	0.040	0.039	0.071	0.039	0.042	0.112	0.111	0.057	0.063	0.095	0.107
		3	0.038	0.039	0.068	0.035	0.037	0.104	0.104	0.060	0.059	0.105	0.097
		4	0.041	0.037	0.066	0.038	0.04	0.109	0.107	0.060	0.058	0.108	0.108
		5	0.039	0.038	0.068	0.037	0.041	0.103	0.104	0.060	0.057	0.107	0.107
		6	0.039	0.037	0.069	0.035	0.038	0.108	0.106	0.059	0.059	0.106	0.103
		7	0.038	0.036	0.069	0.039	0.041	0.110	0.111	0.059	0.059	0.104	0.092
		8	0.037	0.033	0.067	0.033	0.041	0.107	0.108	0.057	0.058	0.103	0.106
		9	0.036	0.036	0.068	0.034	0.034	0.105	0.105	0.057	0.058	0.108	0.108
		10	0.037	0.035	0.071	0.043	0.043	0.103	0.104	0.059	0.060	0.106	0.095
18	Healthy	1	0.043	0.043	0.071	0.036	0.05	0.098	0.099	0.054	0.060	0.104	0.092
		2	0.037	0.042	0.072	0.039	0.039	0.098	0.098	0.055	0.058	0.103	0.104
		3	0.042	0.042	0.071	0.038	0.04	0.108	0.108	0.055	0.055	0.105	0.101
		4	0.040	0.042	0.073	0.034	0.036	0.098	0.099	0.069	0.065	0.093	0.100

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.043	0.048	0.071	0.042	0.045	0.099	0.100	0.056	0.056	0.103	0.102
		6	0.040	0.051	0.070	0.038	0.039	0.098	0.097	0.055	0.055	0.105	0.095
		7	0.042	0.041	0.071	0.038	0.036	0.099	0.099	0.055	0.055	0.108	0.103
		8	0.041	0.043	0.071	0.090	0.041	0.100	0.100	0.055	0.059	0.106	0.103
		9	0.040	0.040	0.071	0.039	0.038	0.097	0.098	0.057	0.056	0.103	0.104
		10	0.042	0.040	0.071	0.043	0.042	0.098	0.098	0.058	0.058	0.104	0.102
19	Healthy	1	0.039	0.039	0.077	0.049	0.049	0.131	0.133	0.058	0.059	0.114	0.109
		2	0.040	0.039	0.082	0.042	0.042	0.104	0.102	0.063	0.066	0.110	0.106
		3	0.040	0.037	0.079	0.051	0.05	0.110	0.109	0.062	0.061	0.109	0.108
		4	0.039	0.043	0.078	0.047	0.046	0.106	0.106	0.064	0.055	0.111	0.100
		5	0.041	0.039	0.079	0.040	0.045	0.104	0.102	0.057	0.062	0.118	0.101
		6	0.048	0.036	0.084	0.050	0.051	0.107	0.108	0.059	0.061	0.110	0.109
		7	0.040	0.046	0.089	0.045	0.047	0.107	0.106	0.068	0.063	0.113	0.105
		8	0.038	0.041	0.084	0.046	0.046	0.111	0.113	0.057	0.059	0.113	0.106
		9	0.032	0.050	0.086	0.047	0.044	0.110	0.111	0.064	0.062	0.115	0.107
		10	0.038	0.037	0.088	0.042	0.044	0.114	0.112	0.068	0.065	0.111	0.107
20	Healthy	1	0.041	0.041	0.070	0.042	0.043	0.100	0.100	0.055	0.056	0.103	0.104
		2	0.040	0.041	0.070	0.036	0.039	0.095	0.095	0.054	0.057	0.097	0.102
		3	0.041	0.040	0.071	0.035	0.031	0.098	0.098	0.061	0.060	0.107	0.108
		4	0.041	0.045	0.072	0.043	0.043	0.099	0.102	0.060	0.058	0.107	0.105
		5	0.043	0.051	0.070	0.038	0.036	0.096	0.097	0.057	0.057	0.104	0.104
		6	0.040	0.042	0.073	0.040	0.040	0.091	0.093	0.060	0.060	0.099	0.101
		7	0.034	0.040	0.071	0.039	0.041	0.094	0.095	0.060	0.059	0.106	0.108
		8	0.038	0.038	0.074	0.035	0.038	0.106	0.104	0.058	0.057	0.102	0.096
		9	0.037	0.043	0.072	0.042	0.043	0.098	0.099	0.056	0.058	0.104	0.101

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.043	0.035	0.071	0.043	0.038	0.094	0.094	0.063	0.078	0.104	0.101
21	MNSV contaminated	1	0.039	0.038	0.071	0.033	0.035	0.103	0.104	0.059	0.060	0.108	0.100
		2	0.039	0.037	0.072	0.036	0.039	0.105	0.105	0.057	0.057	0.105	0.104
		3	0.041	0.045	0.069	0.033	0.039	0.109	0.109	0.058	0.061	0.103	0.104
		4	0.046	0.040	0.073	0.032	0.039	0.107	0.109	0.057	0.057	0.106	0.117
		5	0.044	0.041	0.071	0.035	0.039	0.102	0.104	0.059	0.066	0.107	0.102
		6	0.039	0.038	0.072	0.032	0.038	0.107	0.108	0.057	0.058	0.107	0.107
		7	0.039	0.038	0.069	0.037	0.039	0.107	0.107	0.059	0.057	0.106	0.103
		8	0.041	0.039	0.068	0.034	0.035	0.114	0.112	0.057	0.062	0.113	0.092
		9	0.040	0.038	0.071	0.033	0.033	0.105	0.105	0.062	0.057	0.105	0.103
		10	0.043	0.032	0.072	0.038	0.039	0.105	0.109	0.059	0.059	0.101	0.101
22	SqMV contaminated	1	3.101	3.355	2.045	1.196	1.219	0.586	0.659	0.397	0.303	0.095	0.101
		2	2.111	2.226	1.564	0.21	0.213	0.649	0.709	0.759	0.533	0.688	0.923
		3	0.201	0.177	1.101	0.581	0.605	0.916	0.917	0.098	0.106	0.170	0.214
		4	0.055	0.061	0.893	1.073	1.013	0.981	1.005	0.056	0.057	0.114	0.107
		5	0.044	0.044	1.887	0.17	0.176	0.816	0.776	0.059	0.058	0.112	0.106
		6	0.043	0.047	0.949	1.688	1.797	0.293	0.288	0.058	0.059	2.207	0.718
		7	0.039	0.038	0.668	0.424	0.442	0.123	0.125	0.167	0.178	0.727	0.856
		8	0.037	0.036	0.228	1.794	1.69	0.246	0.251	0.132	0.123	1.234	2.209
		9	0.038	0.036	0.251	0.419	0.436	0.121	0.123	0.054	0.058	0.105	0.103
		10	0.038	0.038	0.918	2.867	2.829	0.155	0.160	0.054	0.056	0.107	0.107
23	Healthy	1	0.039	0.039	0.076	0.052	0.054	0.102	0.102	0.067	0.064	0.124	0.105
		2	0.040	0.040	0.081	0.041	0.039	0.107	0.108	0.068	0.070	0.118	0.097
		3	0.039	0.039	0.081	0.049	0.047	0.106	0.107	0.064	0.063	0.114	0.108
		4	0.039	0.037	0.088	0.042	0.047	0.103	0.103	0.070	0.061	0.114	0.104

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.038	0.039	0.092	0.043	0.046	0.104	0.104	0.066	0.069	0.106	0.115
		6	0.037	0.038	0.088	0.049	0.046	0.110	0.108	0.069	0.074	0.113	0.108
		7	0.036	0.038	0.081	0.047	0.045	0.112	0.114	0.067	0.062	0.111	0.101
		8	0.033	0.039	0.091	0.047	0.047	0.122	0.119	0.059	0.060	0.110	0.101
		9	0.035	0.035	0.081	0.051	0.048	0.104	0.104	0.056	0.064	0.111	0.107
		10	0.036	0.036	0.084	0.043	0.044	0.107	0.108	0.058	0.062	0.115	0.102
24	Healthy	1	0.074	0.074	0.072	0.042	0.038	0.145	0.141	0.058	0.058	0.105	0.104
		2	0.115	0.126	0.083	0.038	0.040	0.098	0.099	0.056	0.057	0.105	0.103
		3	0.148	0.143	0.073	0.035	0.033	0.104	0.101	0.057	0.057	0.100	0.096
		4	0.100	0.099	0.074	0.043	0.041	0.113	0.116	0.059	0.059	0.106	0.106
		5	0.095	0.082	0.070	0.040	0.040	0.116	0.114	0.058	0.057	0.103	0.093
		6	0.121	0.117	0.073	0.041	0.041	0.094	0.095	0.058	0.057	0.104	0.092
		7	0.097	0.100	0.074	0.040	0.038	0.103	0.106	0.058	0.065	0.106	0.104
		8	0.095	0.096	0.073	0.039	0.038	0.105	0.106	0.057	0.065	0.109	0.098
		9	0.062	0.060	0.070	0.044	0.045	0.114	0.112	0.057	0.056	0.106	0.103
		10	0.226	0.223	0.077	0.039	0.037	0.106	0.105	0.056	0.057	0.104	0.104
25	Healthy	1	0.043	0.044	0.076	0.032	0.036	0.105	0.106	0.061	0.054	0.103	0.104
		2	0.044	0.043	0.073	0.036	0.039	0.102	0.103	0.053	0.055	0.104	0.104
		3	0.043	0.044	0.071	0.034	0.037	0.099	0.098	0.061	0.058	0.103	0.103
		4	0.042	0.041	0.074	0.033	0.038	0.110	0.108	0.057	0.056	0.111	0.100
		5	0.043	0.043	0.073	0.035	0.039	0.104	0.105	0.054	0.055	0.107	0.104
		6	0.042	0.042	0.073	0.036	0.038	0.110	0.111	0.060	0.057	0.109	0.103
		7	0.041	0.042	0.072	0.034	0.038	0.108	0.110	0.059	0.057	0.094	0.096
		8	0.040	0.041	0.071	0.037	0.039	0.109	0.112	0.055	0.056	0.093	0.092
		9	0.041	0.042	0.074	0.033	0.036	0.104	0.105	0.060	0.059	0.103	0.094

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.041	0.041	0.072	0.037	0.041	0.102	0.103	0.058	0.059	0.093	0.097
26	SqMV contaminated	1	2.096	2.249	2.191	0.102	0.104	1.201	1.333	0.464	0.403	0.101	0.107
		2	0.138	0.152	1.099	1.422	1.38	0.361	0.388	0.054	0.054	0.459	0.418
		3	0.410	0.447	0.315	0.135	0.14	0.135	0.134	0.058	0.062	0.163	0.208
		4	0.054	0.047	0.125	0.121	0.118	0.101	0.102	0.060	0.060	0.226	0.339
		5	1.726	1.927	0.093	1.493	1.458	0.099	0.099	0.057	0.056	0.376	0.488
		6	0.059	0.060	0.085	0.040	0.044	0.098	0.099	0.058	0.057	0.174	0.219
		7	0.057	0.057	0.082	1.47	1.474	0.103	0.103	0.855	0.746	0.138	0.152
		8	1.870	2.211	0.076	0.103	0.1	0.115	0.117	0.186	0.143	0.120	0.122
		9	0.069	0.073	0.074	0.807	0.812	1.001	1.106	0.055	0.058	0.118	0.111
		10	0.051	0.043	0.071	0.214	0.212	1.021	1.111	0.059	0.063	0.124	0.127
27	MNSV contaminated	1	0.054	0.040	0.072	0.041	0.04	0.107	0.108	0.057	0.055	0.106	0.107
		2	0.039	0.039	0.069	0.042	0.04	0.102	0.103	0.055	0.065	0.102	0.108
		3	0.040	0.042	0.070	0.043	0.042	0.106	0.106	0.056	0.057	0.107	0.106
		4	0.038	0.040	0.068	0.041	0.04	0.101	0.101	0.059	0.055	0.103	0.102
		5	0.037	0.040	0.069	0.035	0.036	0.103	0.103	0.055	0.056	0.103	0.104
		6	0.049	0.041	0.070	0.043	0.041	0.100	0.100	0.057	0.072	0.104	0.107
		7	0.040	0.040	0.068	0.038	0.038	0.103	0.104	0.061	0.054	0.098	0.106
		8	0.040	0.039	0.068	0.040	0.041	0.106	0.107	0.054	0.054	0.106	0.095
		9	0.031	0.038	0.080	0.041	0.04	0.101	0.101	0.055	0.054	0.105	0.105
		10	0.041	0.042	0.070	0.038	0.038	0.098	0.103	0.055	0.061	0.104	0.096
28	Healthy	1	0.046	0.043	0.072	0.043	0.045	0.098	0.099	0.053	0.062	0.105	0.107
		2	0.039	0.044	0.072	0.041	0.039	0.097	0.096	0.060	0.068	0.102	0.104
		3	0.039	0.042	0.074	0.04	0.036	0.100	0.099	0.056	0.056	0.101	0.093
		4	0.037	0.044	0.074	0.043	0.043	0.097	0.096	0.055	0.054	0.094	0.106

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.038	0.050	0.072	0.040	0.041	0.098	0.097	0.060	0.058	0.105	0.098
		6	0.040	0.051	0.072	0.043	0.044	0.093	0.093	0.058	0.055	0.096	0.105
		7	0.037	0.044	0.074	0.044	0.043	0.096	0.097	0.055	0.055	0.095	0.096
		8	0.041	0.041	0.075	0.041	0.041	0.097	0.096	0.057	0.055	0.104	0.103
		9	0.041	0.040	0.072	0.047	0.041	0.097	0.097	0.057	0.057	0.105	0.108
		10	0.036	0.052	0.070	0.042	0.041	0.098	0.098	0.057	0.058	0.103	0.104
29	Healthy	1	0.047	0.040	0.097	0.047	0.052	0.106	0.107	0.061	0.064	0.106	0.094
		2	0.044	0.039	0.084	0.045	0.049	0.114	0.117	0.062	0.064	0.107	0.108
		3	0.038	0.040	0.092	0.037	0.047	0.104	0.103	0.067	0.077	0.112	0.109
		4	0.043	0.039	0.096	0.045	0.051	0.103	0.104	0.063	0.067	0.109	0.108
		5	0.037	0.042	0.093	0.043	0.049	0.100	0.101	0.060	0.063	0.108	0.105
		6	0.036	0.039	0.091	0.042	0.045	0.105	0.104	0.064	0.063	0.105	0.094
		7	0.036	0.039	0.092	0.045	0.048	0.100	0.111	0.063	0.067	0.108	0.098
		8	0.036	0.036	0.089	0.044	0.048	0.105	0.101	0.060	0.060	0.100	0.097
		9	0.036	0.038	0.095	0.043	0.046	0.105	0.106	0.060	0.062	0.096	0.098
		10	0.035	0.037	0.090	0.044	0.046	0.111	0.104	0.060	0.062	0.100	0.108
30	Healthy	1	0.053	0.040	0.098	0.037	0.039	0.112	0.113	0.058	0.065	0.094	0.104
		2	0.041	0.039	0.097	0.040	0.040	0.099	0.100	0.067	0.066	0.108	0.108
		3	0.037	0.038	0.094	0.040	0.040	0.102	0.101	0.064	0.059	0.100	0.094
		4	0.040	0.040	0.097	0.042	0.040	0.095	0.095	0.059	0.056	0.095	0.099
		5	0.035	0.040	0.102	0.041	0.043	0.093	0.093	0.066	0.060	0.110	0.098
		6	0.042	0.034	0.096	0.039	0.039	0.094	0.093	0.061	0.059	0.111	0.107
		7	0.034	0.039	0.097	0.043	0.039	0.099	0.100	0.058	0.057	0.108	0.096
		8	0.045	0.033	0.098	0.044	0.044	0.097	0.097	0.054	0.056	0.111	0.107
		9	0.041	0.041	0.099	0.040	0.04	0.097	0.097	0.055	0.058	0.104	0.105

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.033	0.038	0.097	0.041	0.043	0.099	0.099	0.059	0.060	0.099	0.106

Table E.5. Reproducibility results for the ELISA detection of MNSV. A red cell indicates a positive result. A green cell indicates a negative result.

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	**OD 2**	**OD 1**	**OD 1**	**OD 2**	**OD 1**	**OD 2**	**OD 1**	**OD 2**	**OD 1**	**OD 2**
1	Healthy	1	0.046	0.041	0.063	0.031	0.035	0.112	0.113	0.059	0.061	0.123	0.116
		2	0.044	0.043	0.066	0.033	0.037	0.112	0.113	0.060	0.059	0.119	0.128
		3	0.042	0.042	0.066	0.034	0.039	0.107	0.107	0.056	0.064	0.119	0.121
		4	0.041	0.042	0.064	0.033	0.032	0.117	0.118	0.057	0.050	0.139	0.135
		5	0.046	0.038	0.066	0.034	0.035	0.114	0.116	0.058	0.059	0.122	0.129
		6	0.044	0.037	0.066	0.032	0.037	0.117	0.117	0.059	0.054	0.116	0.107
		7	0.041	0.040	0.064	0.033	0.039	0.124	0.125	0.058	0.058	0.112	0.113
		8	0.041	0.039	0.064	0.034	0.039	0.121	0.121	0.046	0.059	0.113	0.114
		9	0.043	0.036	0.064	0.036	0.036	0.119	0.120	0.059	0.054	0.119	0.114
		10	0.041	0.037	0.066	0.037	0.037	0.126	0.127	0.057	0.056	0.116	0.109
2	Healthy	1	0.045	0.046	0.066	0.038	0.039	0.113	0.113	0.059	0.061	0.141	0.140
		2	0.041	0.042	0.063	0.036	0.038	0.107	0.107	0.061	0.058	0.136	0.150
		3	0.045	0.041	0.064	0.045	0.040	0.114	0.114	0.061	0.057	0.136	0.171
		4	0.043	0.042	0.066	0.031	0.037	0.107	0.107	0.057	0.058	0.130	0.120
		5	0.044	0.042	0.066	0.037	0.038	0.110	0.109	0.060	0.060	0.120	0.125
		6	0.046	0.037	0.065	0.038	0.037	0.107	0.107	0.062	0.063	0.121	0.117
		7	0.041	0.039	0.065	0.036	0.035	0.128	0.128	0.060	0.060	0.111	0.115
		8	0.040	0.037	0.065	0.040	0.039	0.112	0.111	0.060	0.058	0.121	0.128
		9	0.042	0.039	0.064	0.039	0.040	0.111	0.111	0.057	0.060	0.116	0.145
		10	0.041	0.039	0.066	0.039	0.037	0.105	0.106	0.060	0.057	0.134	0.152
3	Healthy	1	0.039	0.039	0.066	0.042	0.041	0.107	0.107	0.055	0.069	0.125	0.112
		2	0.040	0.040	0.067	0.036	0.028	0.112	0.112	0.060	0.062	0.113	0.113
		3	0.038	0.038	0.066	0.041	0.041	0.114	0.115	0.058	0.069	0.114	0.112
		4	0.039	0.036	0.063	0.038	0.038	0.108	0.108	0.056	0.056	0.114	0.102

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.039	0.038	0.064	0.036	0.038	0.114	0.114	0.060	0.059	0.114	0.112
		6	0.037	0.038	0.067	0.040	0.040	0.115	0.115	0.061	0.060	0.112	0.105
		7	0.040	0.037	0.064	0.037	0.039	0.119	0.121	0.057	0.056	0.112	0.114
		8	0.037	0.036	0.064	0.039	0.040	0.123	0.125	0.056	0.069	0.116	0.111
		9	0.037	0.037	0.063	0.040	0.040	0.113	0.113	0.059	0.061	0.122	0.110
		10	0.037	0.036	0.065	0.039	0.038	0.118	0.118	0.058	0.059	0.113	0.113
4	Healthy	1	0.041	0.040	0.063	0.038	0.045	0.105	0.105	0.048	0.087	0.112	0.099
		2	0.039	0.040	0.063	0.038	0.038	0.105	0.106	0.058	0.079	0.106	0.098
		3	0.039	0.039	0.065	0.036	0.034	0.112	0.112	0.066	0.066	0.111	0.110
		4	0.038	0.037	0.064	0.040	0.039	0.121	0.121	0.052	0.058	0.108	0.106
		5	0.039	0.038	0.064	0.040	0.037	0.110	0.110	0.059	0.057	0.110	0.101
		6	0.038	0.037	0.063	0.039	0.040	0.123	0.119	0.056	0.085	0.115	0.110
		7	0.037	0.037	0.065	0.041	0.040	0.104	0.104	0.058	0.059	0.117	0.115
		8	0.036	0.034	0.067	0.037	0.037	0.119	0.118	0.062	0.060	0.111	0.102
		9	0.037	0.035	0.065	0.039	0.034	0.115	0.116	0.057	0.058	0.109	0.097
		10	0.036	0.036	0.065	0.039	0.038	0.111	0.111	0.060	0.063	0.108	0.097
5	Healthy	1	0.085	0.073	0.064	0.032	0.034	0.111	0.111	0.055	0.054	0.117	0.115
		2	0.071	0.067	0.064	0.033	0.035	0.115	0.115	0.054	0.055	0.115	0.113
		3	0.073	0.072	0.063	0.028	0.032	0.127	0.127	0.054	0.053	0.111	0.110
		4	0.077	0.074	0.062	0.033	0.037	0.114	0.114	0.054	0.055	0.112	0.107
		5	0.074	0.075	0.063	0.032	0.032	0.112	0.113	0.056	0.058	0.107	0.097
		6	0.070	0.068	0.064	0.033	0.036	0.119	0.119	0.058	0.062	0.112	0.106
		7	0.074	0.075	0.062	0.032	0.034	0.123	0.122	0.057	0.058	0.110	0.097
		8	0.073	0.067	0.061	0.032	0.031	0.128	0.129	0.056	0.057	0.103	0.108
		9	0.066	0.063	0.063	0.028	0.033	0.118	0.118	0.062	0.060	0.115	0.119

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.045	0.046	0.064	0.035	0.046	0.117	0.117	0.062	0.060	0.117	0.109
6	Healthy	1	0.041	0.040	0.063	0.032	0.034	0.113	0.113	0.063	0.058	0.113	0.112
		2	0.039	0.039	0.062	0.036	0.037	0.112	0.113	0.059	0.061	0.113	0.113
		3	0.040	0.037	0.064	0.034	0.037	0.105	0.105	0.058	0.059	0.110	0.111
		4	0.039	0.039	0.065	0.034	0.036	0.102	0.103	0.059	0.057	0.107	0.107
		5	0.041	0.040	0.063	0.037	0.036	0.124	0.125	0.060	0.059	0.105	0.099
		6	0.039	0.037	0.063	0.035	0.033	0.108	0.108	0.071	0.061	0.116	0.111
		7	0.037	0.037	0.064	0.036	0.034	0.105	0.104	0.059	0.058	0.124	0.123
		8	0.037	0.038	0.066	0.040	0.037	0.110	0.109	0.058	0.062	0.137	0.126
		9	0.036	0.036	0.064	0.036	0.036	0.107	0.107	0.056	0.057	0.129	0.120
		10	0.036	0.034	0.061	0.040	0.039	0.110	0.110	0.059	0.066	0.112	0.112
7	Healthy	1	0.039	0.039	0.065	0.040	0.039	0.115	0.115	0.068	0.092	0.114	0.110
		2	0.039	0.038	0.063	0.040	0.036	0.112	0.113	0.060	0.057	0.118	0.112
		3	0.040	0.036	0.062	0.041	0.039	0.118	0.119	0.056	0.064	0.112	0.098
		4	0.037	0.038	0.062	0.039	0.036	0.111	0.111	0.060	0.060	0.119	0.099
		5	0.042	0.037	0.063	0.034	0.036	0.144	0.143	0.062	0.061	0.119	0.122
		6	0.038	0.037	0.063	0.038	0.040	0.114	0.114	0.059	0.055	0.113	0.120
		7	0.037	0.037	0.062	0.037	0.038	0.111	0.110	0.075	0.065	0.117	0.122
		8	0.036	0.036	0.061	0.038	0.038	0.120	0.120	0.058	0.063	0.112	0.114
		9	0.037	0.036	0.063	0.037	0.040	0.109	0.109	0.058	0.059	0.113	0.111
		10	0.036	0.036	0.063	0.036	0.037	0.114	0.114	0.058	0.059	0.118	0.114
8	SqMV contaminated	1	0.044	0.042	0.063	0.038	0.045	0.110	0.111	0.060	0.065	0.120	0.125
		2	0.043	0.042	0.062	0.034	0.035	0.106	0.106	0.058	0.063	0.120	0.118
		3	0.042	0.039	0.066	0.035	0.034	0.136	0.137	0.057	0.063	0.133	0.119
		4	0.042	0.040	0.064	0.039	0.039	0.117	0.117	0.064	0.059	0.140	0.143

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.043	0.038	0.062	0.035	0.037	0.112	0.111	0.068	0.056	0.128	0.152
		6	0.043	0.037	0.061	0.039	0.039	0.107	0.108	0.058	0.058	0.122	0.126
		7	0.041	0.037	0.064	0.038	0.039	0.113	0.113	0.060	0.059	0.120	0.138
		8	0.042	0.034	0.064	0.039	0.038	0.110	0.110	0.065	0.060	0.125	0.123
		9	0.040	0.034	0.063	0.037	0.038	0.115	0.115	0.060	0.057	0.123	0.125
		10	0.038	0.040	0.062	0.036	0.037	0.109	0.109	0.058	0.060	0.125	0.128
9	SqMV contaminated	1	0.043	0.040	0.062	0.032	0.035	0.143	0.145	0.062	0.061	0.130	0.135
		2	0.041	0.041	0.064	0.035	0.039	0.112	0.112	0.060	0.057	0.140	0.167
		3	0.041	0.040	0.062	0.035	0.037	0.114	0.113	0.056	0.058	0.112	0.125
		4	0.043	0.039	0.063	0.035	0.038	0.131	0.128	0.057	0.064	0.113	0.125
		5	0.041	0.041	0.063	0.034	0.038	0.123	0.122	0.062	0.058	0.125	0.122
		6	0.041	0.038	0.064	0.029	0.037	0.114	0.117	0.057	0.056	0.128	0.130
		7	0.041	0.040	0.062	0.035	0.037	0.121	0.121	0.057	0.060	0.133	0.136
		8	0.040	0.036	0.062	0.036	0.038	0.121	0.121	0.061	0.056	0.106	0.130
		9	0.038	0.038	0.062	0.033	0.034	0.128	0.127	0.057	0.057	0.113	0.120
		10	0.039	0.038	0.063	0.037	0.036	0.125	0.124	0.056	0.061	0.108	0.120
10	MNSV contaminated	1	0.174	0.203	0.240	0.341	0.321	0.212	0.215	0.102	0.084	0.182	0.212
		2	0.147	0.155	0.196	0.149	0.147	0.206	0.208	0.069	0.082	0.415	0.457
		3	0.157	0.142	0.128	0.234	0.244	0.318	0.322	0.164	0.132	0.435	0.414
		4	0.106	0.110	0.251	0.242	0.232	0.300	0.303	0.104	0.134	1.213	1.333
		5	0.090	0.092	0.185	0.156	0.159	0.176	0.177	0.122	0.111	0.234	0.259
		6	0.146	0.147	0.141	0.144	0.144	0.298	0.304	0.214	0.186	0.266	0.291
		7	0.104	0.110	0.129	0.632	0.629	0.252	0.255	0.130	0.130	0.506	0.479
		8	0.131	0.109	0.199	0.258	0.257	0.235	0.238	0.124	0.087	0.260	0.280
		9	0.088	0.083	0.173	0.148	0.143	0.213	0.215	0.068	0.093	0.283	0.271

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.088	0.076	0.165	0.162	0.156	0.498	0.505	0.140	0.250	0.253	0.248
11	SqMV contaminated	1	0.040	0.040	0.062	0.041	0.041	0.113	0.114	0.059	0.060	0.111	0.126
		2	0.039	0.039	0.065	0.036	0.034	0.114	0.116	0.058	0.057	0.110	0.133
		3	0.036	0.040	0.064	0.040	0.040	0.118	0.119	0.062	0.061	0.133	0.119
		4	0.041	0.038	0.064	0.040	0.038	0.117	0.116	0.059	0.055	0.116	0.117
		5	0.038	0.040	0.063	0.036	0.035	0.118	0.120	0.057	0.057	0.114	0.109
		6	0.035	0.038	0.064	0.038	0.040	0.119	0.118	0.059	0.058	0.113	0.115
		7	0.038	0.038	0.062	0.035	0.038	0.124	0.125	0.060	0.057	0.113	0.114
		8	0.031	0.036	0.062	0.035	0.040	0.126	0.126	0.060	0.058	0.118	0.120
		9	0.037	0.036	0.062	0.038	0.038	0.122	0.122	0.056	0.057	0.114	0.119
		10	0.036	0.035	0.066	0.033	0.038	0.127	0.126	0.058	0.059	0.126	0.131
12	Healthy	1	0.039	0.039	0.062	0.039	0.038	0.110	0.110	0.059	0.064	0.107	0.113
		2	0.039	0.038	0.062	0.036	0.036	0.106	0.105	0.058	0.059	0.107	0.107
		3	0.038	0.036	0.063	0.034	0.032	0.108	0.108	0.057	0.066	0.100	0.106
		4	0.039	0.035	0.064	0.038	0.038	0.113	0.114	0.066	0.055	0.108	0.098
		5	0.037	0.037	0.062	0.035	0.039	0.119	0.120	0.055	0.058	0.112	0.111
		6	0.036	0.036	0.062	0.038	0.039	0.107	0.107	0.056	0.058	0.108	0.108
		7	0.035	0.034	0.062	0.037	0.037	0.106	0.107	0.060	0.062	0.108	0.120
		8	0.035	0.034	0.064	0.037	0.036	0.107	0.108	0.052	0.058	0.111	0.117
		9	0.035	0.033	0.062	0.040	0.037	0.103	0.104	0.057	0.063	0.104	0.112
		10	0.036	0.034	0.062	0.036	0.036	0.123	0.123	0.058	0.057	0.113	0.109
13	SqMV contaminated	1	0.040	0.041	0.063	0.033	0.036	0.114	0.114	0.065	0.072	0.108	0.106
		2	0.040	0.040	0.071	0.033	0.036	0.117	0.117	0.063	0.096	0.108	0.116
		3	0.039	0.039	0.066	0.034	0.037	0.109	0.109	0.067	0.078	0.139	0.129
		4	0.039	0.040	0.064	0.033	0.037	0.106	0.106	0.062	0.060	0.124	0.126

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.041	0.042	0.063	0.034	0.039	0.113	0.114	0.080	0.063	0.120	0.121
		6	0.037	0.037	0.066	0.033	0.037	0.139	0.139	0.076	0.064	0.129	0.114
		7	0.036	0.036	0.063	0.032	0.037	0.121	0.120	0.076	0.063	0.108	0.113
		8	0.038	0.034	0.063	0.033	0.037	0.112	0.112	0.059	0.066	0.112	0.116
		9	0.035	0.035	0.065	0.032	0.035	0.109	0.109	0.060	0.074	0.119	0.116
		10	0.036	0.037	0.063	0.038	0.039	0.107	0.107	0.062	0.075	0.117	0.140
14	MNSV contaminated	1	0.160	0.134	0.140	0.239	0.240	0.431	0.435	0.236	0.263	0.242	0.262
		2	0.241	0.270	0.166	0.281	0.299	0.217	0.218	0.089	0.087	0.269	0.320
		3	0.214	0.216	0.208	0.120	0.121	0.307	0.309	0.078	0.082	0.392	0.389
		4	0.098	0.084	0.149	0.179	0.189	0.425	0.426	0.117	0.092	0.295	0.328
		5	0.151	0.173	0.305	0.506	0.468	0.162	0.162	0.160	0.136	0.209	0.201
		6	0.330	0.352	0.377	0.399	0.411	0.268	0.269	0.174	0.140	0.389	0.400
		7	0.115	0.133	0.373	0.164	0.159	0.530	0.535	0.159	0.208	0.463	0.408
		8	0.114	0.138	0.289	0.382	0.378	0.697	0.702	0.193	0.260	0.320	0.319
		9	0.100	0.108	0.226	0.198	0.221	0.264	0.265	0.191	0.237	0.321	0.343
		10	0.139	0.126	0.202	0.125	0.119	0.350	0.357	0.096	0.101	0.345	0.378
15	SqMV contaminated	1	0.041	0.040	0.064	0.043	0.044	0.110	0.111	0.059	0.053	0.112	0.125
		2	0.040	0.040	0.065	0.040	0.040	0.113	0.113	0.053	0.059	0.117	0.117
		3	0.040	0.039	0.064	0.040	0.037	0.122	0.122	0.059	0.060	0.111	0.111
		4	0.038	0.039	0.063	0.039	0.038	0.117	0.118	0.060	0.054	0.122	0.119
		5	0.042	0.037	0.064	0.030	0.034	0.115	0.115	0.053	0.056	0.119	0.113
		6	0.039	0.037	0.065	0.040	0.041	0.109	0.107	0.059	0.058	0.112	0.116
		7	0.036	0.038	0.065	0.035	0.038	0.114	0.113	0.060	0.058	0.132	0.157
		8	0.036	0.038	0.064	0.038	0.036	0.117	0.118	0.056	0.059	0.121	0.138
		9	0.034	0.037	0.064	0.041	0.044	0.122	0.125	0.059	0.060	0.119	0.124

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.040	0.043	0.065	0.035	0.038	0.110	0.110	0.058	0.058	0.120	0.121
16	Healthy	1	0.040	0.037	0.062	0.036	0.037	0.105	0.105	0.054	0.055	0.107	0.108
		2	0.038	0.038	0.064	0.035	0.035	0.106	0.106	0.053	0.056	0.112	0.114
		3	0.037	0.038	0.063	0.032	0.031	0.107	0.107	0.059	0.056	0.102	0.107
		4	0.037	0.037	0.064	0.038	0.038	0.106	0.106	0.056	0.054	0.110	0.108
		5	0.038	0.038	0.063	0.037	0.036	0.109	0.109	0.059	0.066	0.109	0.116
		6	0.037	0.037	0.063	0.037	0.037	0.110	0.110	0.058	0.061	0.114	0.117
		7	0.039	0.032	0.064	0.039	0.038	0.106	0.106	0.056	0.060	0.110	0.111
		8	0.034	0.036	0.066	0.036	0.035	0.114	0.114	0.052	0.058	0.109	0.109
		9	0.037	0.033	0.061	0.037	0.039	0.117	0.117	0.055	0.059	0.108	0.120
		10	0.036	0.036	0.061	0.037	0.035	0.115	0.115	0.055	0.056	0.117	0.111
17	MNSV contaminated	1	0.309	0.350	0.198	0.123	0.128	0.200	0.203	0.192	0.191	0.259	0.288
		2	0.081	0.086	0.138	0.144	0.151	0.154	0.154	0.124	0.118	0.393	0.417
		3	0.098	0.090	0.218	0.234	0.224	0.364	0.373	0.206	0.181	0.342	0.365
		4	0.097	0.092	0.158	0.122	0.120	0.461	0.470	0.161	0.129	0.221	0.288
		5	0.121	0.122	0.263	0.454	0.441	1.241	1.279	0.074	0.071	0.448	0.507
		6	0.076	0.075	0.170	0.237	0.240	0.281	0.284	0.130	0.136	0.256	0.254
		7	0.232	0.234	0.134	0.178	0.175	0.211	0.215	0.104	0.096	0.282	0.289
		8	0.155	0.126	0.113	0.154	0.149	0.445	0.458	0.119	0.115	0.507	0.578
		9	0.825	0.635	0.124	0.201	0.196	0.242	0.246	0.093	0.096	0.357	0.479
		10	0.089	0.075	0.176	0.173	0.174	0.401	0.410	0.129	0.122	0.589	0.501
18	Healthy	1	0.039	0.038	0.062	0.035	0.036	0.110	0.108	0.056	0.056	0.107	0.097
		2	0.038	0.036	0.062	0.037	0.038	0.108	0.107	0.056	0.054	0.106	0.096
		3	0.038	0.036	0.063	0.035	0.038	0.115	0.115	0.054	0.055	0.108	0.098
		4	0.037	0.036	0.064	0.035	0.036	0.111	0.111	0.081	0.054	0.096	0.102

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.037	0.037	0.063	0.038	0.038	0.106	0.106	0.054	0.058	0.107	0.106
		6	0.038	0.035	0.063	0.037	0.038	0.105	0.105	0.066	0.059	0.103	0.108
		7	0.035	0.037	0.062	0.037	0.036	0.112	0.112	0.058	0.069	0.112	0.099
		8	0.033	0.037	0.063	0.038	0.041	0.120	0.120	0.057	0.061	0.109	0.109
		9	0.035	0.034	0.062	0.039	0.039	0.116	0.116	0.056	0.060	0.126	0.111
		10	0.034	0.034	0.063	0.040	0.037	0.104	0.104	0.057	0.055	0.107	0.106
19	Healthy	1	0.046	0.041	0.063	0.040	0.040	0.124	0.124	0.058	0.057	0.114	0.121
		2	0.044	0.039	0.065	0.037	0.034	0.112	0.112	0.060	0.052	0.117	0.108
		3	0.041	0.040	0.063	0.041	0.040	0.115	0.115	0.061	0.058	0.133	0.127
		4	0.041	0.038	0.064	0.039	0.038	0.109	0.109	0.058	0.056	0.136	0.131
		5	0.040	0.041	0.065	0.035	0.035	0.112	0.111	0.059	0.065	0.136	0.108
		6	0.039	0.039	0.068	0.038	0.040	0.141	0.141	0.062	0.060	0.110	0.127
		7	0.040	0.039	0.064	0.038	0.037	0.118	0.117	0.061	0.060	0.114	0.119
		8	0.039	0.037	0.064	0.038	0.040	0.117	0.117	0.059	0.063	0.110	0.109
		9	0.038	0.038	0.064	0.038	0.038	0.115	0.115	0.065	0.060	0.119	0.105
		10	0.038	0.038	0.066	0.034	0.036	0.114	0.114	0.059	0.058	0.119	0.120
20	Healthy	1	0.039	0.039	0.061	0.038	0.040	0.115	0.114	0.054	0.056	0.106	0.108
		2	0.039	0.038	0.061	0.037	0.036	0.111	0.110	0.055	0.053	0.109	0.101
		3	0.039	0.037	0.062	0.036	0.034	0.109	0.109	0.054	0.055	0.113	0.104
		4	0.039	0.035	0.062	0.040	0.039	0.105	0.105	0.055	0.053	0.106	0.108
		5	0.039	0.035	0.062	0.035	0.036	0.107	0.107	0.054	0.053	0.108	0.103
		6	0.037	0.037	0.061	0.038	0.039	0.103	0.103	0.054	0.054	0.103	0.105
		7	0.037	0.037	0.062	0.038	0.037	0.118	0.119	0.054	0.056	0.108	0.109
		8	0.035	0.035	0.062	0.037	0.036	0.121	0.121	0.054	0.055	0.107	0.106
		9	0.037	0.033	0.060	0.038	0.038	0.116	0.116	0.052	0.056	0.105	0.108

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.035	0.035	0.061	0.037	0.036	0.110	0.110	0.055	0.057	0.108	0.110
21	MNSV contaminated	1	0.144	0.155	0.172	0.253	0.255	0.185	0.188	0.076	0.080	0.410	0.508
		2	0.103	0.111	0.120	0.271	0.263	0.458	0.466	0.066	0.068	0.295	0.324
		3	0.090	0.105	0.115	0.133	0.140	0.248	0.248	0.264	0.227	0.421	0.420
		4	0.214	0.237	0.096	0.152	0.149	0.220	0.221	0.136	0.116	0.187	0.166
		5	0.091	0.083	0.150	0.123	0.129	0.203	0.193	0.133	0.131	0.395	0.324
		6	0.093	0.092	0.173	0.169	0.182	0.212	0.212	0.096	0.087	0.208	0.190
		7	0.197	0.165	0.153	0.098	0.097	0.200	0.205	0.104	0.110	0.321	0.372
		8	0.158	0.158	0.136	0.300	0.301	0.612	0.615	0.062	0.062	0.268	0.266
		9	0.122	0.112	0.113	0.167	0.167	0.245	0.247	0.075	0.074	0.217	0.196
		10	0.079	0.088	0.200	0.234	0.244	0.446	0.454	0.118	0.105	0.360	0.375
22	SqMV contaminated	1	0.042	0.040	0.063	0.034	0.037	0.110	0.110	0.060	0.076	0.100	0.107
		2	0.042	0.039	0.063	0.039	0.040	0.109	0.108	0.078	0.082	0.116	0.109
		3	0.041	0.037	0.063	0.037	0.038	0.102	0.102	0.086	0.092	0.142	0.117
		4	0.040	0.039	0.064	0.036	0.037	0.232	0.232	0.059	0.056	0.124	0.117
		5	0.040	0.038	0.063	0.038	0.038	0.102	0.102	0.072	0.073	0.118	0.114
		6	0.038	0.037	0.062	0.033	0.038	0.104	0.103	0.062	0.067	0.114	0.099
		7	0.038	0.036	0.064	0.037	0.037	0.110	0.110	0.074	0.085	0.105	0.112
		8	0.037	0.037	0.064	0.042	0.042	0.112	0.111	0.059	0.057	0.111	0.114
		9	0.036	0.037	0.062	0.039	0.038	0.115	0.115	0.062	0.078	0.106	0.110
		10	0.037	0.034	0.063	0.041	0.041	0.106	0.106	0.069	0.057	0.113	0.117
23	Healthy	1	0.044	0.042	0.063	0.041	0.041	0.111	0.112	0.061	0.061	0.112	0.109
		2	0.042	0.040	0.065	0.039	0.035	0.114	0.115	0.062	0.060	0.127	0.135
		3	0.042	0.038	0.063	0.040	0.038	0.120	0.120	0.058	0.058	0.117	0.127
		4	0.040	0.040	0.065	0.035	0.039	0.118	0.118	0.068	0.060	0.120	0.118

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.044	0.039	0.070	0.034	0.037	0.103	0.103	0.060	0.060	0.110	0.104
		6	0.041	0.041	0.067	0.040	0.040	0.116	0.116	0.059	0.058	0.109	0.105
		7	0.039	0.039	0.064	0.036	0.036	0.116	0.116	0.058	0.056	0.113	0.117
		8	0.039	0.037	0.065	0.039	0.039	0.115	0.113	0.058	0.057	0.119	0.118
		9	0.037	0.038	0.063	0.037	0.040	0.116	0.116	0.055	0.058	0.110	0.125
		10	0.040	0.038	0.064	0.037	0.038	0.151	0.152	0.061	0.058	0.112	0.125
24	Healthy	1	0.059	0.057	0.063	0.037	0.038	0.120	0.120	0.057	0.059	0.108	0.105
		2	0.080	0.078	0.063	0.034	0.032	0.117	0.117	0.061	0.064	0.105	0.096
		3	0.081	0.080	0.062	0.034	0.030	0.121	0.122	0.059	0.059	0.106	0.107
		4	0.069	0.069	0.063	0.041	0.039	0.122	0.122	0.060	0.054	0.109	0.108
		5	0.061	0.058	0.062	0.032	0.032	0.110	0.111	0.058	0.059	0.108	0.098
		6	0.082	0.080	0.065	0.038	0.040	0.125	0.126	0.061	0.061	0.099	0.112
		7	0.079	0.070	0.062	0.038	0.039	0.117	0.117	0.060	0.056	0.122	0.117
		8	0.064	0.065	0.063	0.035	0.035	0.179	0.181	0.055	0.062	0.101	0.111
		9	0.047	0.046	0.061	0.037	0.039	0.121	0.122	0.063	0.058	0.107	0.109
		10	0.083	0.084	0.062	0.035	0.034	0.178	0.180	0.068	0.060	0.107	0.107
25	Healthy	1	0.040	0.041	0.063	0.029	0.034	0.137	0.138	0.054	0.057	0.184	0.108
		2	0.040	0.040	0.064	0.032	0.037	0.108	0.109	0.050	0.055	0.109	0.108
		3	0.038	0.040	0.065	0.029	0.035	0.197	0.199	0.064	0.056	0.097	0.097
		4	0.039	0.040	0.063	0.030	0.036	0.112	0.113	0.055	0.054	0.109	0.113
		5	0.039	0.040	0.062	0.031	0.035	0.112	0.112	0.056	0.054	0.118	0.110
		6	0.037	0.040	0.065	0.030	0.036	0.114	0.113	0.056	0.054	0.103	0.113
		7	0.037	0.038	0.062	0.029	0.036	0.115	0.115	0.057	0.059	0.101	0.099
		8	0.036	0.037	0.063	0.031	0.040	0.114	0.114	0.053	0.054	0.099	0.108
		9	0.036	0.037	0.064	0.029	0.034	0.242	0.246	0.052	0.056	0.108	0.107

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.038	0.036	0.065	0.037	0.037	0.111	0.110	0.054	0.056	0.112	0.098
26	SqMV contaminated	1	0.042	0.042	0.062	0.037	0.036	0.131	0.132	0.070	0.060	0.138	0.119
		2	0.040	0.039	0.064	0.037	0.035	0.105	0.106	0.058	0.063	0.150	0.135
		3	0.041	0.041	0.063	0.037	0.038	0.109	0.109	0.067	0.062	0.184	0.173
		4	0.040	0.041	0.064	0.037	0.038	0.105	0.105	0.066	0.086	0.132	0.131
		5	0.039	0.041	0.063	0.039	0.039	0.111	0.111	0.054	0.059	0.140	0.125
		6	0.042	0.037	0.065	0.038	0.039	0.108	0.109	0.063	0.065	0.126	0.128
		7	0.039	0.040	0.063	0.039	0.035	0.107	0.107	0.063	0.062	0.144	0.119
		8	0.038	0.037	0.065	0.042	0.041	0.111	0.111	0.075	0.058	0.141	0.125
		9	0.038	0.039	0.063	0.040	0.039	0.107	0.107	0.057	0.062	0.138	0.110
		10	0.040	0.040	0.062	0.041	0.041	0.148	0.141	0.058	0.064	0.130	0.132
27	MNSV contaminated	1	0.147	0.144	0.234	0.326	0.321	0.336	0.345	0.140	0.159	0.338	0.601
		2	0.096	0.100	0.166	0.152	0.146	0.471	0.482	0.086	0.083	0.228	0.506
		3	0.165	0.146	0.149	0.411	0.400	0.333	0.339	0.072	0.091	0.176	0.169
		4	0.078	0.073	0.230	0.176	0.175	0.180	0.183	0.141	0.124	0.394	0.426
		5	0.082	0.089	0.213	0.485	0.476	0.201	0.205	0.067	0.070	0.255	0.401
		6	0.119	0.120	0.177	0.172	0.169	0.233	0.237	0.160	0.173	0.410	0.598
		7	0.078	0.079	0.138	0.202	0.219	0.370	0.375	0.077	0.060	0.179	0.212
		8	0.083	0.078	0.141	0.294	0.293	0.196	0.198	0.066	0.073	0.343	0.451
		9	0.076	0.100	0.108	0.208	0.214	0.330	0.338	0.091	0.061	0.343	0.443
		10	0.072	0.131	0.192	0.199	0.204	0.220	0.223	0.064	0.077	0.272	0.243
28	Healthy	1	0.048	0.043	0.062	0.036	0.039	0.110	0.110	0.071	0.056	0.112	0.107
		2	0.040	0.040	0.062	0.036	0.038	0.103	0.103	0.056	0.057	0.109	0.108
		3	0.040	0.039	0.062	0.035	0.036	0.110	0.110	0.058	0.056	0.107	0.103
		4	0.040	0.037	0.064	0.039	0.040	0.106	0.107	0.060	0.057	0.105	0.099

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		5	0.041	0.038	0.062	0.037	0.037	0.110	0.110	0.058	0.058	0.114	0.112
		6	0.038	0.038	0.063	0.038	0.039	0.111	0.110	0.066	0.059	0.111	0.098
		7	0.037	0.038	0.062	0.038	0.038	0.108	0.108	0.061	0.056	0.101	0.109
		8	0.036	0.039	0.064	0.037	0.036	0.104	0.106	0.056	0.056	0.107	0.106
		9	0.038	0.036	0.063	0.038	0.038	0.111	0.111	0.057	0.056	0.114	0.105
		10	0.036	0.038	0.062	0.038	0.037	0.107	0.107	0.057	0.057	0.102	0.094
29	Healthy	1	0.042	0.047	0.063	0.032	0.037	0.090	0.090	0.058	0.057	0.114	0.100
		2	0.043	0.045	0.063	0.035	0.039	0.106	0.106	0.060	0.061	0.120	0.112
		3	0.042	0.041	0.063	0.034	0.037	0.114	0.115	0.061	0.062	0.117	0.116
		4	0.042	0.043	0.063	0.033	0.039	0.111	0.112	0.063	0.056	0.110	0.113
		5	0.041	0.043	0.063	0.034	0.039	0.110	0.109	0.057	0.056	0.112	0.104
		6	0.040	0.040	0.063	0.034	0.039	0.120	0.119	0.062	0.062	0.110	0.102
		7	0.039	0.041	0.062	0.032	0.039	0.111	0.111	0.062	0.061	0.126	0.109
		8	0.043	0.036	0.062	0.033	0.039	0.112	0.112	0.054	0.055	0.123	0.119
		9	0.041	0.037	0.063	0.034	0.038	0.118	0.117	0.056	0.051	0.114	0.112
		10	0.040	0.040	0.064	0.042	0.040	0.115	0.114	0.056	0.056	0.112	0.111
30	Healthy	1	0.042	0.043	0.062	0.032	0.033	0.138	0.140	0.058	0.061	0.103	0.106
		2	0.041	0.041	0.063	0.035	0.038	0.193	0.195	0.057	0.056	0.240	0.110
		3	0.041	0.041	0.063	0.036	0.036	0.162	0.164	0.057	0.069	0.167	0.107
		4	0.041	0.041	0.064	0.037	0.038	0.166	0.168	0.057	0.059	0.110	0.105
		5	0.042	0.039	0.063	0.035	0.037	0.175	0.170	0.059	0.057	0.111	0.111
		6	0.041	0.041	0.062	0.033	0.035	0.169	0.171	0.057	0.062	0.120	0.117
		7	0.039	0.040	0.066	0.037	0.036	0.172	0.174	0.059	0.055	0.110	0.115
		8	0.038	0.041	0.066	0.042	0.042	0.175	0.177	0.060	0.068	0.112	0.111
		9	0.044	0.043	0.062	0.036	0.034	0.106	0.107	0.060	0.059	0.105	0.108

Lot	Sample status	Subsample	Laboratory 2		Laboratory 3	Laboratory 4		Laboratory 5		Laboratory 6		Laboratory 7	
			OD 1	OD 2	OD 1	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2	OD 1	OD 2
		10	0.040	0.038	0.063	0.041	0.039	0.165	0.167	0.057	0.057	0.111	0.108