APPENDIX I – RECOMMENDED PROCEDURES FOR DETECTION OF VIRUSES OF SMALL FRUIT CROPS

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The following tables outline the procedures recommended for the detection of viruses of small fruit crops in the genera, *Fragaria*, *Humulus*, *Ribes*, *Rubus* and *Vaccinium*. In cases where a test needs validation it is recommended that an additional test be performed on the test plants. These tests that need validation have only been used on one or a few isolates of a virus. Before they can be recommended tests they need to be evaluated on a broad range of isolates from multiple locations. In the case of polymerase chain reaction assays it is necessary to state primer sequences that have been shown to be useful for detecting a wide range of virus isolates when recommending a test. Only a subset of a virus sequence is likely to be highly conserved and suitable for detecting all strains of a virus. This is also true for monoclonal antibodies that will be recommended for ELISA tests. Some monoclonal antibodies will be strain specific as has been shown for tomato ringspot, tobacco streak, cucumber mosaic etc. In some cases, polyclonal antisera must be designated as well since strain specificity can be a problem with some polyclonal antibodies, this is notably so with many nepoviruses.

At the meetings in Valencia, Spain in 2003, it was decided that a standard protocol should be used to validate new testing procedures before they are listed as recommended procedures. This protocol would apply to new biological indicators, serological assays or nucleic acid based assays. The data set that will be required before a new test is recommended is given in the Proceedings of the Tree Fruit Symposium from the meetings in Spain. Data sets for new test protocols will be presented at future symposia of the Small Fruit Working Group of the ISHS and the decision to accept the new tests will be made at the symposia. Acceptable tests will then be added to the list of recommended procedures.

Fragaria species (Strawberry)
Humulus species (Hops)
Ribes species (Currants and Gooseberry)
Rubus species (Raspberry, Blackberry and Hybrid Berries)
Vaccinium species (Blueberry, Cranberry and Lingonberry)

STRAWBERRY INDEXING PROCEDURES

Agent/Disease	BioAssays[a]	Laboratory Tests	Tests that need validation[b]	Refs
Arabis mosaic	*C. quinoa*	ELISA		1,2
Aster yellows phytoplasma		PCR	ELISA	1,2,3,4,7 9,10
Fragaria chiloensis	Cucumber	ELISA		16
Raspberry ringspot	*C. quinoa*	ELISA[c]		1,2
Strawberry chlorotic fleck	EMK			1,2
Strawberry crinkle	UC-5, -6, 'Alpine'		PCR	1,2,13
Strawberry feather leaf	'Alpine'			1,2
Strawberry green petal phytoplasma		PCR	ELISA	1,2,3,4,7 9, 10
Strawberry latent C	UC-5, EMC			1,2
Strawberry latent ringspot	*C. quinoa*	ELISA		1,2,11
Strawberry leafroll	UC-5, -10			1,2
Strawberry lethal decline	'Alpine'		PCR	1,2,3,4,7 9,10
Strawberry marginal chlorosis			PCR	12
Strawberry mild yellow edge	UC-4, -5, 'Alpine' Negative on UC-6	ELISA	PCR	1,2,6, 8,14
Strawberry mottle	UC-5, 'Alpine'		PCR, ELISA	1,2,15
Strawberry mycoplasma			PCR	1,2,34,7

yellows				9,10
Strawberry pallidosis	UC-10, -11		DsRNA, PCR	1,2,18,19 21
Strawberry pseudo mild yellow-edge	UC-4, -12, 'Alpine'	ELISA		1,2,22
Strawberry rickettsia yellows				1,2
Strawberry veinbanding	UC-5, -6, 12 'Alpine'		PCR, ELISA	1,2,5, 17
Tobacco necrosis	*C. quinoa*	ELISA		1,2
Tobacco streak (Strawberry necrotic shock)	*C. quinoa*	ELISA[c]		1,2,20
Tomato black ring	*C. quinoa*	ELISA[c]		1,2
Tomato ringspot	*C. quinoa*	ELISA[c]		1,2

[a] Sap and graft transmissions should be done in the early spring and one should use young vigorous indicator plants for graft assays. BioAssay by sap transmission is less reliable than ELISA tests and if possible ELISA testing should be done to confirm negative BioAssay results.

[b] Tests that need validation. These tests reported in the scientific literature to be able to detect the given pathogen, however, at this time only one or a few isolates of the pathogen have been studied. A broader range of isolates need to be detected with the described assay to ensure its usefulness in detecting a broad range of isolates of the pathogen before the test can be recommended for certification or quarantine purposes.

[c] Indicates virus is quite variable and a single antiserum may not detect all isolates. This is especially true if one is using monoclonal antibodies.

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HOPS INDEXING PROCEDURES

Agent/Disease	BioAssay[a]	Laboratory Assays	Tests that need validation[b]	Ref.
American hop latent	*C. quinoa*	ELISA		2
Apple mosaic	Cucumber	ELISA		7
Arabis mosaic Hop bare bine, Hop chlorotic disease, Hop nettle head, Hop split leaf blotch	*C. quinoa*	ELISA		3
Hop latent viroid		cDNA Hybrdization	PCR	8
Hop latent carlavirus		ELISA		2,6
Hop mosaic		ELISA		1,5
Hop stunt viroid	Cucumber	cDNA Hybridizatioin	PCR	8,9,10
Humulus japonicus virus	*C. quinoa*	ELISA		4
Prunus necrotic ringspot	Cucumber	ELISA		7

[a] Sap transmission should be done in the early spring. BioAssay by sap transmission is less reliable than ELISA tests and if possible ELISA testing should be done to confirm negative BioAssay results.

[b]Tests that need validation. These tests reported in the scientific literature to be able to detect the given pathogen, however, at this time only one or a few isolates of the pathogen have been studied. A broader range of isolates need to be detected with the described assay to ensure its usefulness in detecting a broad range of isolates of the pathogen before the test can be recommended for certification or quarantine purposes.

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CURRANTS AND GOOSEBERRIES INDEXING PROCEDURES

Agent/Disease	BioAssays[a]	Laboratory Assays	Tests that need validation[b]	Refs
Alfalfa mosaic Interveinal white mosaic	*C. quinoa*	ELISA		1,3
Arabis mosaic Black currant yellow mottle	*C. quinoa*	ELISA		1,6
Black currant gold dust				1
Black currant reversion	'Amos Black', 'Baldwin', 'Ojebyn'		PCR, ELISA	1,4,5
Black currant vein clearing	'Amos Black' B1385/81 (gooseberry)			1
Black currant yellows	'Amos Black'			1
Cucumber mosaic Black currant green mottle	*C. quinoa*	ELISA[c]		1,2
Gooseberry veinbanding	'Amos Black' B1385/81 (gooseberry		PCR	1
Infectious variegation				1
Raspberry ringspot Gooseberry deterioration, Red currant spoon leaf	*C. quinoa*	ELISA*		1,8
Red currant full blossom phytoplasma	'Houghton Castle' (red currant)		PCR	1
Red currant yellow leaf spot	'Laxton No. 1', 'Fay's Prolific' (red currant)			1
Strawberry latent ringspot	*C. quinoa*	ELISA		1,7

Tomato black ring	*C. quinoa*			9
Tomato ringspot	*C. quinoa*			10

[a] Sap and graft transmissions should be done in the early spring and one should use young vigorous indicator plants for graft assays. BioAssay by sap transmission is less reliable than ELISA tests and if possible ELISA testing should be done to confirm negative BioAssay results.

[b]Tests that need validation. These tests reported in the scientific literature to be able to detect the given pathogen, however, at this time only one or a few isolates of the pathogen have been studied. A broader range of isolates need to be detected with the described assay to ensure its usefulness in detecting a broad range of isolates of the pathogen before the test can be recommended for certification or quarantine purposes.

[c] Indicates virus is quite variable and a single antiserum may not detect all isolates. This is especially true if one is using monoclonal antibodies.

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RASPBERRY AND BLACKBERRY INDEXING PROCEDURES

Agent/Disease	BioAssays[a]	Laboratory Tests	Tests that need validation[b]	Refs
Apple mosaic	*C. quinoa*	ELISA		3,4
Arabis mosaic	*C. quinoa*	ELISA		3,4
Blackberry calico disease, Wineberry latent	*C. quinoa*, 'Marionberry'		ELISA	3,4,6
Black raspberry necrosis	*C. quinoa*, *R. occidentalis*			3,4
Boysenberry decline	'Boysenberry'		PCR	3
Bramble yellow mosaic	*C. quinoa*			3,4
Cherry leaf roll	*C. quinoa*	ELISA[c]		3,4
Cherry rasp leaf	*C. quinoa*	ELISA		3,4
Cucumber mosaic	*C. quinoa*	ELISA[c]		3,4
Hawaiian rubus leaf curl	*R.macraei*			
Raspberry bushy dwarf	*C. quinoa*	ELISA		3,4
Raspberry chlorotic net disease, raspberry vein chlorosis				3,4
Raspberry leaf curl	*R. occidentalis* *R. phoenicolasius*			3,4
Raspberry leaf mottle	*R. idaeus* 'Malling Landmark'			3,4
Raspberry leaf spot	*R. idaeus* 'Norfolk Giant'			3,4
Raspberry mosaic disease complex,	*R. occidentalis*			3,4

Raspberry ringspot	*C. quinoa* *C. amaranticolor*	ELISA[c]		3,4
Raspberry spot mosaic disease (see Raspberry leaf spot and Raspberry leaf mottle)				3,4
Raspberry veinbanding mosaic complex (see also Raspberry leaf mottle)	*R. idaeus* 'Malling Jewel'			3,4,5
Raspberry vein chlorosis	*R. idaeus* 'Delight'			3,4
Raspberry witches broom, Rubus stunt	*R. idaeus* 'Lloyd George'	PCR		3,4
Raspberry yellow spot	*R. idaeus* 'Malling Promise'			2,4
Rubus chinese seed-borne	*C. quinoa*	ELISA		1,4
Rubus yellow net	*R. occidentalis,* *R. macraei*		PCR	3,4,5,7
Strawberry latent ringspot	*C. quinoa*	ELISA		3,4
Thimbleberry ringspot	*R. parviflorus*			3,4
Tobacco ringspot	*C. quinoa*	ELISA[c]		3,4
Tobacco streak Black raspberry latent	*C. quinoa*	ELISA[c]		3,4
Tomato black ring	*C. quinoa*	ELISA[c]		3,4
Tomato ringspot	*C. quinoa*	ELISA[c]		3,4
Wineberry latent	*C. quinoa*		ELISA	3,4,6

[a] Sap and graft transmissions should be done in the early spring and one should use young vigorous indicator plants for graft assays. BioAssay by sap transmission is less reliable than ELISA tests and if possible ELISA testing should be done to confirm negative BioAssay results.

[b] Tests that need validation. These tests reported in the scientific literature to be able to detect

the given pathogen, however, at this time only one or a few isolates of the pathogen have been studied. A broader range of isolates need to be detected with the described assay to ensure its usefulness in detecting a broad range of isolates of the pathogen before the test can be recommended for certification or quarantine purposes.

[c] Indicates virus is quite variable and a single antiserum may not detect all isolates. This is especially true if one is using monoclonal antibodies.

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BLUEBERRY INDEXING PROCEDURES

Agent/Disease	BioAssay[a]	Laboratory Assays	Tests that need validation[b]	Refs
Blueberry leaf mottle	*C. quinoa*	ELISA		1,2,4
Blueberry mosaic			PCR	1,2,6
Blueberry red ringspot		ELISA	PCR	1,2
Blueberry scorch		ELISA[c]	PCR	1,3,4,5
Blueberry shock	*N. clevelandii*	ELISA	PCR	1,2,4
Blueberry shoestring		ELISA		1,2,4
Blueberry stunt	'Cabot'	DAPI,	PCR	1,2
Cranberry false blossom	None		PCR	1,2
Cranberry ringspot	None			1,2
Peach rosette mosaic	*C. quinoa*	ELISA		1,2
Tobacco ringspot	*C. quinoa*	ELISA		1,2,4
Tomato ringspot	*C. quinoa*	ELISA[d]		1,2,4

[a] Sap transmission from blueberry should be done in the early spring, preferably using flower tissue. BioAssay by sap transmission is less reliable than ELISA tests and if possible ELISA testing should be done to confirm negative BioAssay results.

[b]Tests that need validation. These tests reported in the scientific literature to be able to detect the given pathogen, however, at this time only one or a few isolates of the pathogen have been studied. A broader range of isolates need to be detected with the described assay to ensure its usefulness in detecting a broad range of isolates of the pathogen before the test can be recommended for certification or quarantine purposes.

[c] Antiserum available for blueberry scorch virus work well with isolates from the Pacific

Northwest but less well with isolates from New Jersey (formerly known as Sheep Pen Hill).

[d]Tomato ringspot virus is quite variable and a single antiserum may not detect all isolates. This is especially true if one is using monoclonal antibodies.

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