

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2012.12103

AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

Technical Brief

Lateral Flow Devices to Rapidly Determine Levels of Stable *Botrytis* Antigens in Table and Dessert Wines

Frances M. Dewey,^{1*} Christopher C. Steel,² and Sarah J. Gurr³

¹Research Associate, ³Professor, Plant Sciences, University of Oxford, South Parks Rd., Oxford, OX1 3RB, UK; and ²Professor, National Wine and Grape Industry Centre, School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.

*Corresponding author (email: molly@fmdewey.com)

Acknowledgments: This research was supported by a Senior Fellowship award to Prof Chris Steel from the British Society of Plant Pathology. The authors also thank Ms. C.E. Hofmann for collating the collection of dessert wines used in this study. The hybridoma cell line secreting the *Botrytis* monoclonal antibody, BC-12.C A4, employed in the Lateral Flow devices, is the property of Oxford University and is used under licence from the University by Forsite Pocket Diagnostics, UK, and EnviroLogix, US. Some royalties go to author F.M. Dewey.

Manuscript submitted Aug 2012, revised Dec 2012, accepted Dec 2012

Copyright © 2012 by the American Society for Enology and Viticulture. All rights reserved.

Abstract: Two commercially available *Botrytis* Lateral Flow Devices (B-LFDs) (immunochromatographic devices), one from EnviroLogix, Maine, USA the other from Forsite Diagnostics, York, UK were tested and compared for their ability to detect and quantify levels of a highly stable *Botrytis* antigen in table wines and dessert wines. Table wines were diluted 1:40 and dessert wines 1:500 in phosphate buffered saline plus Tween 20 (0.05% v/v) (PBST). Results from both types of devices were comparable and repeatability was good. This study shows that *Botrytis* Lateral Flow Devices could provide a useful tool for wine makers interested in relating levels of *Botrytis* antigens in table and dessert wines to sensory properties.

Key words: antigens, *Botrytis*, grey mould, Lateral Flow devices, noble rot, table and dessert wines

27

Introduction

28 It is well recognized that *Botrytis cinerea* is the main cause of bunch rot of grape berries in
29 temperate climates (Marois et al. 1993, Slomczynski et al. 1995). Bunch rot is undesirable for production
30 of table wines because it is associated with off-flavours and unpleasant aromas in finished wines and loss
31 of colour in red wines. In contrast, the presence of *B. cinerea* infections in late harvest grape berries is
32 desirable because *Botrytis* is believed to be responsible for the desirable aromas and flavours (Sarrazin et
33 al. 2007, Sivertsen et al. 2005). However, in both cases, any study of the relationship between levels of
34 *Botrytis* in wines and sensory properties of wines is difficult because there has been no easy way to
35 measure levels of *Botrytis* antigens in finished wines. The use of rapid, user-friendly, immunodiagnostic
36 Lateral-Flow Devices offers an easy method to detect fungal antigens (Dewey et al. 2008). The
37 advantages of LFDs over other immunological methods, such as microtitre plate ELISAs (Dewey et al.
38 2000; Dewey 2002), and molecular tests are that they are rapid, (5-15min), can be easily operated by
39 untrained workers and do not require laboratory facilities.

40 Two commercially available B-LFDs have been developed for the detection and quantification of
41 soluble stable *Botrytis*-antigens, one produced by EnviroLogix, Portland, ME, USA and the other by
42 Forsite Pocket Diagnostics, York, UK. Although both employ the same *Botrytis* monoclonal antibody,
43 BC-12.CA4 (Meyer and Dewey 2000), that recognizes a constitutively produced, thermostable antigen,
44 that is not metabolized during fermentation, there are technical differences and the performance of both
45 tests has never been compared.

46 The format used by both devices is a sandwich assay in which the antigens are captured by the
47 *Botrytis* antibody which is bound, in the EnviroLogix test to gold nanoparticles and, in the Pocket
48 Diagnostics test to 'latex' particles. The bound conjugates are present in an absorbent pad overlaying a

49 nitrocellulose membrane (Fig 1). When the test liquid is applied, the particles move along the membrane
50 by capillary action; those carrying the *Botrytis* antigens are arrested at the test line on the membrane by a
51 pre-printed line of *Botrytis*-antibodies. In the EnviroLogix test, the positive test line appears pink because
52 the antibody is bound to gold particles whereas the positive test line in the Forsite Pocket Diagnostics
53 devices appears blue because the latex-antibody conjugate is blue. Those particles to which no antigen is
54 bound continue to move forward along the membrane and, in the case of the EnviroLogix test they are
55 arrested at the control line, by a pre-printed line of anti-mouse antibodies, to give a second pink line. The
56 control line in the Pocket Diagnostics test is formed in a different way. Blue latex particles, coated with
57 non-specific rabbit-antibodies, are mixed with the murine *Botrytis*-bound latex particles in the absorbent
58 starting pad and these, which are not arrested at the test line, are arrested at the control line by a line of
59 anti-rabbit antibodies (Fig. 2). The differences between the two types of LFDs, apart from colour of the
60 test and control bands, could be significant. The control line in the EnviroLogix tests is dependent on
61 there being an excess of *Botrytis* gold-conjugates particles binding at the control line whereas the Pocket
62 Diagnostics control line is not dependent on an excess of *Botrytis* antibody coated latex particles. These
63 technical differences mean that, if excess antigen is present, there could be significant differences in the
64 intensity of the control bands because the SI value is the percentage of the reflectivity of the background
65 minus that of the test line divided by the reflectivity of the background minus that of the control line. The
66 reflectivities of the test lines, control lines and backgrounds are measured with their respective custom-
67 made scanners (reflectometers). The percentage of the reflectivity of the background minus that of the
68 test line divided by the reflectivity of the control line is automatically expressed by the scanners as the
69 Signal Intensity (SI).

70 The aims of this study were to compare two commercially available B-LFDs for the
71 quantification of *Botrytis* antigens in a range of red and white table wines and dessert wines.

72

Materials and Methods

73

74

75

76

Wines. All wines tested were purchased either in the UK or Australia or, in the case of the reference dessert wine Dolce, from Far Niente, California, USA. Details of grape varietal and vintage of the wines are given for red wines in Table 1, white wines in Table 2, dessert wines in Table 3. All wines were stored and tested at room temperature, precipitates were not seen in any of the red wines.

77

78

79

80

81

82

83

84

85

86

87

88

89

90

Lateral Flow Devices. B-LFD kits and their respective scanners were purchased from EnviroLogix (kit batch number 151208) (Portland, ME, USA) and from Forsite Pocket Diagnostics (kit batch number Y01 for table wines and AA01 for desert wines) (York, UK). The two different batches of Forsite Pocket Diagnostic kits differed in that AA01 had a higher concentration of the *Botrytis* antibody conjugated particles in the test pad than Y01. Unless otherwise stated, all wines were diluted into phosphate buffered saline + Tween 20 (0.05% v/v) (PBST), table wines were diluted 1:40, dessert wines 1:500. Tests with the EnviroLogix B-LFDs were done by placing the absorbent pad of the device in 400µl of diluted wine for 10 min; the absorbent pad was then removed and the device was inserted into the EnviroLogix reader (scanner) to determine the SI value. For the Forsite Pocket Diagnostics tests, 70 µl of the diluent were pipetted into the well of the device and, after 10 min, the devices were inserted into the Forsite reader and the SI value recorded. On completion the SI values of the EnviroLogix tests remained stable because the absorbent start pad is removed before the SI values are determined but the SI values of the Pocket Diagnostics tests tend to increase with time because the start pad is not removed. Therefore, care was taken to time the tests and determine the SI values in both cases after 10 min.

91

92

93

To determine repeatability of the devices and threshold detection levels a dilution series of the reference dessert wine Dolce (Far Niente, CA, USA, 1998 vintage), used in previous studies (Dewey et al. 2008) was tested.

Results and Discussion

94

95 **Standards and Repeatability** In tests, done with both devices, on a series of dilutions of the
96 reference dessert wine, Dolce, correlation was good, R^2 0.939, (Fig 3). The problem of repeatability was
97 addressed in a previous publication by Dewey et al, (2008) where tests were done in triplicate on a
98 dilution series of the dessert wine Dolce. There was little variation between replicates and so for this
99 reason, and to reduce total costs of the project, tests on each of the experimental table and dessert wines
100 were only done once. The threshold detections levels were similar. Both devices could detect down to
101 0.0125% standard in PBST but there were differences in the numerical value of the signals from the
102 control or buffer alone, SI values for the EnviroLogix devices were zero, but for the Forsite devices they
103 ranged from 0 to 6 (batch Y01) and from 18.6 to 20 (batch AA01).

104

105 **Table wines.** SI values from the tests done on 36 red and 32 white table wines and 27 dessert wines are
106 given in Tables 1, 2, and 3, respectively. In all cases, there was good correlation between the SI values
107 from the EnviroLogix B-LFDs and the Forsite Pocket Diagnostics B-LFDs (Figs. 4A, B and C For red
108 wines, the R^2 value was 0.867 for white wines 0.7456 and 0.849 for dessert wines. The SI values from
109 tests on red wines by the EnviroLogix B-LFDs ranged from 0 to 48 and from 3.3 to 36 by the Forsite
110 Diagnostics B-LFDs. In tests on white wines, SI values ranged from 0 to 44 with the EnviroLogix B-
111 LFDs and from 3.3 to 36 with the Forsite Pocket Diagnostics LFDs. The range in SI values from tests
112 with the EnviroLogix B-LFDs was greater than those with the Forsite Pocket Diagnostics LFDs; this, in
113 the case of the high-end results, probably reflects the difference in the capture antibodies used for the
114 control lines. At high concentrations of antigen, the *Botrytis* binding sites on the antibody-coated particles
115 in the test pad of both devices become saturated. This does not affect the intensity of the control line in
116 the Forsite devices because the formation of the control line is independent of the level of antigen

117 binding. But, in the EnviroLogix devices the control line is dependent upon there being an excess of free,
118 unbound *Botrytis* antibody-coated nanogold particles. Therefore, when there are fewer *Botrytis*-free
119 antibody bound particles available to travel to the control line the intensity of the control line is lower. In
120 both cases, because the SI value is calculated from the percentage difference between the reflectance of
121 the background minus the test line divided by the reflectance of the background minus the control line, a
122 weaker control line would give higher SI values.

123 **Dessert wines.** The SI values using the Forsite B-LFD (batch AA01) were much higher than
124 those from Envirologix B-LFDs. Although the values were higher (15 to 134) they correlated well with
125 the SI values from the EnviroLogix B-LFDs (0-50) giving an R^2 value of 0.849 (Fig. 4C).

126 **Applications.** Both devices are simple and easy to use. The Forsite Pocket Diagnostics devices
127 and their custom made reader (scanner) are designed to be used in the field. Their devices, which are
128 encased in plastic, are more robust but more expensive.

129 Pilot studies, not reported here, have shown that the level of *Botrytis*-antigens in grape juice
130 remains constant throughout fermentation irrespective of grape varietal. These devices should therefore,
131 provide useful tools for relating levels of *Botrytis* antigens to the sensory properties of wines such as taste
132 and aroma and could also be of particular value for sparkling wine production where proteases produced
133 by *Botrytis* are known to affect the formation and retention of bubbles (Marchal et al. 2001, Cilindre et al.
134 2007).

135 We did not attempt to relate the SI values from tests on finished wines to levels of rot in the
136 vineyard. Previous studies have shown that the *Botrytis*-SI values correlate well with tests on juice from
137 hand-picked, hand-sorted, berries but not with visual estimates of machine-picked grapes (Dewey et al.
138 2008). In the latter, heavily-infected berries are often not counted because they are easily squashed

139 during transit. On-site field estimates of rot are also problematic because they are totally dependent on
140 the method of sampling and the variations found within different climatic regions in vineyards. We found
141 that, in tests with juices, diluted 1:40 in PBST, from hand-picked, hand-sorted grape berries (Chardonnay)
142 with 0.6, 1.3, 2.5, 5.0 and 10.0% *Botrytis*- rot (determined on the basis of incidence, not weight) that SI
143 values with the EnviroLogix B-LFDs were 10.5, 16.7, 26.2, 34.6 and 39.7 respectively (Dewey et al.,
144 2008). Thus, B-LFD tests on red and white table wines with SI values greater than 35 indicate that the
145 wines were made from grape berries with 5% or more average level of *Botrytis* rot. However, the value of
146 the *Botrytis* LFDs is not in determining estimates of rot in the field but in providing a means of measuring
147 the levels of *Botrytis* antigens throughout the fermentation process and in finished wines.

148 **Conclusions**

149 Both the EnviroLogix and the Forsite Pocket Diagnostics *Botrytis* LFDs are useful tools for rapidly
150 determining levels of *Botrytis* antigens in wines. They provide an easy mechanism for wine makers
151 interested in studying the relationship between of the levels of *Botrytis* antigens and the aroma and
152 flavours of wines. Results from both devices were comparable. The Forsite Pocket Diagnostics B-LFDs
153 and their custom-made reader are designed for field use; they are more expensive than the EnviroLogix
154 B-LFDs but, because they are housed in plastic they have the advantage of being more robust.

155 **Literature Cited**

- 156 Cilindre, C., A.J. Castro, C. Clement, P. Jeandet, and R. Marchal. 2007. Food Chemistry 103:139-149.
- 157 Dewey, F.M., S.E. Ebeler, D.O. Adams, A.C. Noble, and U.M. Meyer. 2000. Quantification of *Botrytis*
158 in grape juice determined by a monoclonal antibody-based immunoassay. American Journal of
159 Viticulture and Enology 51:276-282.
- 160 Dewey, F.M. 2002. *Botrytis* antigens in wine. The Australian and New Zealand Grapegrower and
161 Winemaker, March issue, 20-21.

- 162 Dewey, F.M., M. Hill, And R. De Scenzo. 2008. Quantification of *Botrytis* and Laccase in Wine grapes.
163 American Journal of Enology and Viticulture 59: 47-54.
- 164 Marchal, R., I. Tabary, M. Valade, D. Moncomble, L. Viaux, B. Robillard, and P. Jeandet. 2001 Effects
165 of *Botrytis cinerea* infection on Champagne wine foaming properties. Journal of Science Food and
166 Agriculture 10: 1371-20001.
- 167 Marois, J.J, A.M. Bledsoe, R.W. Ricker, and R.M. Bostock. 1993. Sampling for *Botrytis cinerea* in
168 harvested grape berries. American Journal of Enology and Viticulture 44: 261-265.
- 169 Meyer, U. and F.M. Dewey. 2000. Efficacy of different immunogens for raising monoclonal antiobides
170 to *Botrytis cinerea*. Mycological Research 104: 979-987.
- 171 Sarrazin, E., D. Dubourdieu, and P. Darriet. 2007. Characterization of key-aroma compounds of
172 botrytized wines, influence of grape botrytization. Food Chemistry 103: 536-545.
- 173 Slomczynski, D., J.P. Nakas, and S.W. Tanenbaum. 1995. Production and characterisation of laccase from
174 *Botrytis cinerea*. Applied Environmental Microbiology 61: 907-912.
- 175 Sivertsen, H.K., F.M. Dewey, and H. Heymann. 2005. Relationship between sensory descriptive analysis
176 and levels of *Botrytis* antigens in dessert wines. American Journal of Enology and Viticulture 56: 330-
177 335.
- 178

Table 1 Red wines tested for levels of *Botrytis* antigens by EnviroLogix (EL) and Forsite Pocket Diagnostics (FPD) *Botrytis* Lateral Flow devices. SI = signal intensity. Wines were diluted 1/40 in PBST prior to analysis. Each wine was only tested once.

Variety	Origin	Vintage	SI EL	SI FPD
Barbera	Italy	2007	27	18.1
Cab Sauv	Australia	2006	0	5.2
Cab Sauv	Australia	2009	0	4.8
Cab Sauv / Merlot	Australia	2008	0	6.3
Cab Sauv / Merlot	Chile	2009	5	5.9
Cab Sauv / Merlot	France	2005	10	6.3
Carmenere	Chile	Unknown	9	6.8
Grenache / Tempranillo	Spain	2009	18	10.3
Malbec	Argentina	2008	5	5.6
Malbec	Argentina	Unknown	14	9.1
Merlot	Chile	2009	7	3.3
Merlot / Cab Sauv	France	2007	28	15.3
Monastrell	Spain	2003	18	10.6
Monastrell	Spain	2005	37	18.5
Pinot noir	Argentina	2008	40	24.3
Pinot noir	Australia	2005	23	13.4
Pinot noir	Australia	2008	0	5.2
Pinot noir	Australia	2006	0	6.6
Rioja	Spain	2003	32	20.6
Rioja	Spain	2005	35	19
Rioja	Spain	2007	35	16.6
Rioja	Spain	2008	36	17.6
Shiraz	Australia	2008	0	3.4
Shiraz	Australia	2009	4	4.9
Syrah	France	2007	30	17.9
Unknown	France -Beaujolais	2007	35	15.9
Unknown	France -Bordeaux	1988	10	6.8
Unknown	France -Bordeaux	1988	48	35.5
Unknown	France -Bordeaux	1998	48	34.3
Unknown	France -Bordeaux	2005	5	3.2
Unknown	France -Bordeaux	2007	29	17
Unknown	France -Cotes du Rhone	2001	8	6.9
Unknown	Italy -Chianti	1998	38	20.1
Unknown	Italy -Chianti	2006	22	10.8
Unknown	Portugal -Alentejo	2008	5	6.2
Unknown	Spain -Madeira	1981	29	11.3

Cab Sauv = Cabernet Sauvignon.

Table 2 White wines tested for levels of *Botrytis* antigens by EnviroLogix (EL) and Forsite Pocket Diagnostics (FPD) *Botrytis* Lateral Flow devices. SI = signal intensity. Wines were diluted 1/40 in PBST prior to analysis. Each wine was only tested once.

Variety	Origin	Vintage	SI EL	SI FPD
Chardonnay	Australia	2007	13	7.2
Chardonnay	Australia	2008	0	3.5
Chardonnay	Australia	2008	7	7.8
Chardonnay	Australia	2009	0	7.1
Chardonnay	Australia	2009	0	4.0
Chardonnay	Australia	2008	4	10.0
Chardonnay	Chile	2009	8	4.7
Chardonnay	France	2007	41	26.6
Chardonnay	France	2008	30	16.4
Chardonnay	France	2008	44	28.5
Chardonnay	S. Africa	2008	8	7.7
Chardonnay	S. Africa	2009	6	5.5
Chardonnay	S. Africa	2009	9	7.1
Chardonnay	Australia	2008	43	29.2
Chardonnay/Viognier	France	2009	0	14.2
Gewurztraminer	Chile	2008	9	0.7
Muscat	France	2008	29	8.2
Petit Chablis	France	2008	38	22.4
Pinot Grigio	Italy	2009	15	8.6
Pinot Grigio	Italy	2009	16	7.7
Pinot Grigio	NZ	2008	0	7.2
Pinot Grigio	Italy	2009	11	9.1
Pouilly Fume	France	2008	29	14.9
Riesling	Australia	2008	2	5.6
Riesling	Germany	2007	32	18.7
Riesling	Germany	2007	28	20.4
Riesling	NZ	2008	34	23.4
Sancerre	French	2007	29	14.0
Sauvignon Blanc	Chile	2009	16	6.7
Sem. / Sauv. Blanc	Australia	2009	11	3.6
UWB	France	2008	9	9.4
Viognier	Argentina	2009	7	5.4

UWB = Unknown white blend

Table 3 Dessert wines tested for levels of *Botrytis* antigens by EnviroLogix (EL) and Forsite Pocket Diagnostics (FPD) *Botrytis* Lateral Flow devices. Wines were diluted 1/500 in PBST prior to analysis. SI = signal intensity. Bottle rep = bottle replicates of the same wine/vintage. Each bottle was only tested once. All wines of Australian origin were made with Semillon grapes while wines from France were made with an unknown blend of Semillon and Sauvignon Blanc.

Wine	Bottle rep	Origin	Vintage	SI EL	FPD
1	1	Australia	1984	45	100
2	1	Australia	1985	40	82
3	1	Australia	1995	45	96
4	1	Australia	2000	41	106
5	1	Australia	2002	34	79
6	1	Australia	2004	25	71
6	2	Australia	2004	22	61
6	3	Australia	2004	25	79
6	4	Australia	2004	27	66
7	1	Australia	2004	30	57
7	2	Australia	2004	29	59
7	3	Australia	2004	30	68
8	1	Australia	2005	14	32
9	1	Australia	2006	0	15
9	2	Australia	2006	0	17
9	3	Australia	2006	0	14
10	1	Australia	2006	24	86
10	2	Australia	2006	25	57
10	3	Australia	2006	22	44
11	1	Australia	2006	5	32
11	2	Australia	2006	5	34
11	3	Australia	2006	6	35
12	1	Australia	2006	35	62
12	2	Australia	2006	36	88
12	3	Australia	2006	27	68
13	1	Australia	2006	23	85
13	2	Australia	2006	16	36
13	3	Australia	2006	16	42
13	4	Australia	2006	24	46
14	1	Australia	2007	13	44
14	2	Australia	2007	17	53
14	3	Australia	2007	16	44
15	1	Australia	2007	33	75
15	2	Australia	2007	29	75
15	3	Australia	2007	33	92
16	1	Australia	2008	42	100
16	2	Australia	2008	47	98
16	3	Australia	2008	47	126
17	1	Australia	2008	47	89
17	2	Australia	2008	46	115
18	1	Australia	2008	36	72
18	2	Australia	2008	33	94
18	3	Australia	2008	36	73
18	4	Australia	2008	35	74

Table 3 continued

Wine	Bottle rep	Origin	Vintage	SI EL	FPD
19	1	Australia	2009	42	97
19	2	Australia	2009	44	104
19	3	Australia	2009	44	114
20	1	Australia	unknown	46	104
21	1	Australia	unknown	50	118
22	1	Australia	unknown	49	134
23	1	Australia	unknown	36	86
24	1	France	2001	13	47
24	2	France	2001	15	53
24	3	France	2001	17	34
25	1	France	2004	23	32
25	2	France	2004	25	64
25	3	France	2004	25	61
26	1	France	2005	17	48
26	2	France	2005	16	35
26	3	France	2005	15	35
27	1	France	unknown	18	61

Flow of particles when diluted wine sample added to start pad

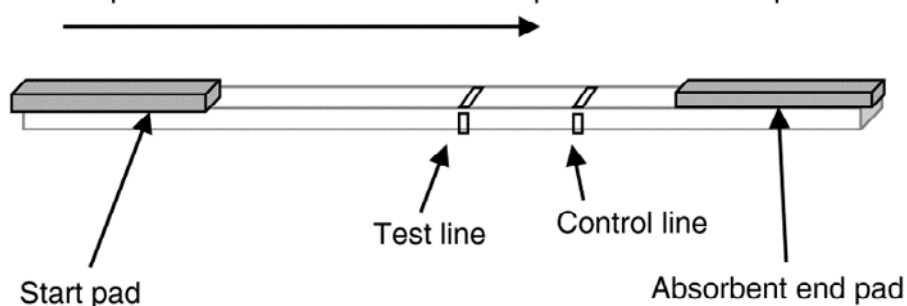


Figure 1 Diagram of Lateral Flow Device. The absorbent start pad in EnviroLogix devices contains *Botrytis* antibodies bound to nanogold particles and the control line is a pre-printed line of anti-mouse antibodies. In the Forsite Diagnostics devices the absorbent start pad contains two sets of particles one to which *Botrytis* antibodies are bound and the other to which non-specific rabbit antibodies are bound; the control line is composed of a pre-printed line non-specific anti-rabbit antibodies.

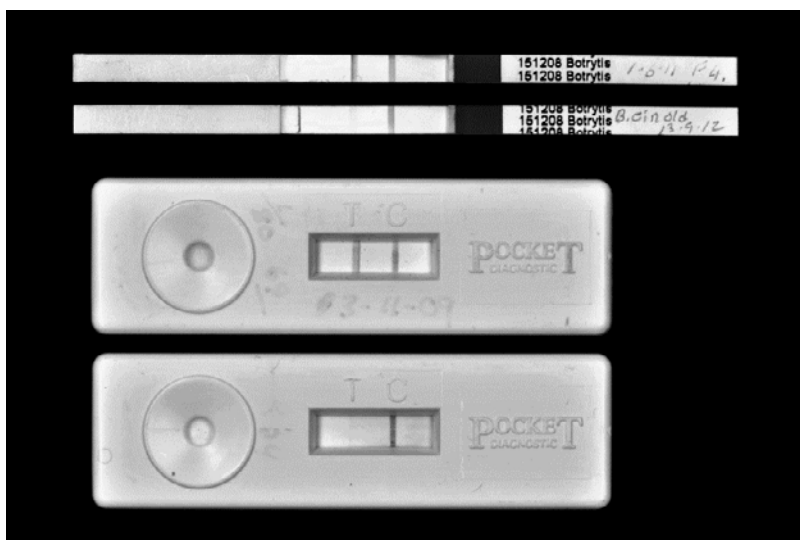


Figure 2 Completed Lateral-Flow tests, (a) EnviroLogix tests, (b) Forsite-Pocket Diagnostics tests: + positive test result, - negative test result.

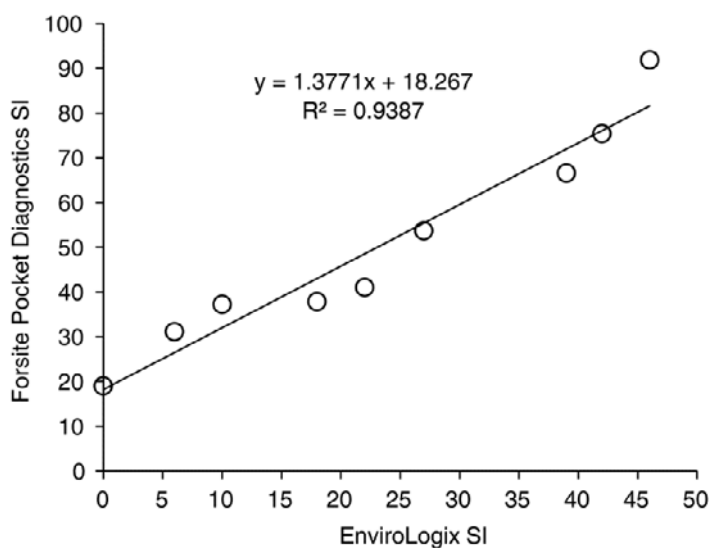


Figure 3 Comparison of the EnviroLogix B-LFDs with the Forsite Pocket Diagnostics B-LFDs (batch U05) for the quantification of *Botrytis* antigens in a dilution series (0.0 - 1%) of the reference dessert wine, Dolce, in PBST, SI= Signal intensity.

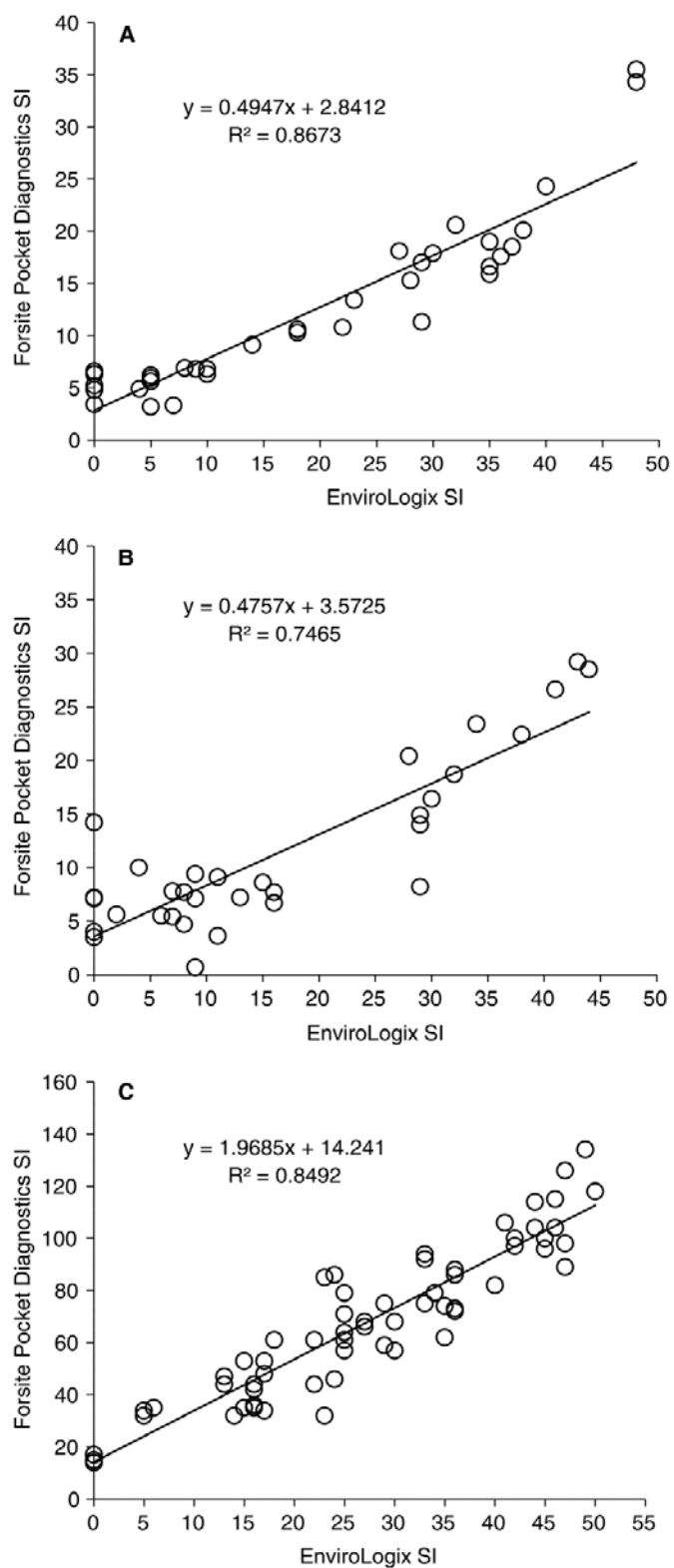


Figure 4 Comparison of the EnviroLogix B-LFD with the Forsite Pocket Diagnostics B-LFD for the quantification of *Botrytis* antigens in red wines (A), white wines (B), and a series of dessert wines from Australia and France (C). SI= Signal intensity.