

Supplementary Information

Supplementary Table S1. Assessment of the diagnostic agreement between either the PrioCHECK® or IDvet FMDV NS ELISAs and the in-house ELISA tests for detecting infection and/or carrier status in unvaccinated, vaccinated, bovine NSP panel, and cattle field sera. κ = kappa statistic; PPA = positive agreement (%); NPA = negative agreement (%).

	Prionics			IDvet Long			IDvet Short		
	κ	PPA	NPA	κ	PPA	NPA	κ	PPA	NPA
<i>Unvaccinated Infected Recovered</i>									
2B	0.87	88.68	99.19	0.82	88.68	99.19	0.85	90.38	99.19
3B	0.82	88.68	98.37	0.85	88.68	98.37	0.86	90.38	98.38
3ABC	0.87	88.68	99.19	0.82	88.68	99.19	0.87	90.38	99.19
3D	0.81	86.79	97.26	0.82	86.79	97.26	0.85	90.38	97.36
3CD	0.81	86.79	98.56	0.83	86.79	98.58	0.85	88.46	98.58
2C	0.17	75.47	76.85	0.12	73.58	76.75	0.13	75.00	76.77
<i>Unvaccinated Carrier</i>									
2B	0.86	78.57	99.19	0.87	78.57	99.19	0.88	81.48	99.19
3B	0.88	78.57	98.37	0.88	78.57	98.37	0.86	81.48	98.38
3ABC	0.86	78.57	99.19	0.87	78.57	99.19	0.88	81.48	99.19
3D	0.84	75.00	97.26	0.85	75.00	97.26	0.86	81.48	97.36
3CD	0.86	75.00	98.58	0.87	75.00	98.58	0.88	77.78	98.58
2C	0.06	57.14	76.85	0.06	53.57	76.75	0.07	55.55	76.77
<i>Vaccinated Infected Recovered</i>									
2B	0.74	67.58	98.85	0.77	73.30	98.11	0.76	72.86	98.57
3B	0.85	84.02	98.18	0.84	85.86	97.08	0.82	85.43	97.62
3ABC	0.77	69.86	99.14	0.79	75.92	98.49	0.79	74.37	98.76
3D	0.67	70.32	95.31	0.66	72.25	94.34	0.65	71.86	94.78
3CD	0.66	63.01	97.22	0.66	64.92	96.42	0.65	65.33	96.96
2C	0.18	47.03	77.01	0.19	49.21	76.72	0.22	49.25	76.92
<i>Vaccinated Carrier</i>									
2B	0.77	71.72	98.90	0.76	73.38	98.71	0.77	73.68	98.32
3B	0.81	79.31	98.20	0.81	82.73	98.21	0.83	82.71	97.73
3ABC	0.81	75.17	99.20	0.81	76.26	98.91	0.80	78.19	98.71
3D	0.64	62.76	97.00	0.63	64.03	96.82	0.64	64.66	96.54
3CD	0.64	57.93	98.30	0.62	58.99	98.11	0.64	58.65	97.73
2C	0.17	50.34	76.80	0.16	50.36	76.64	0.17	50.37	76.48
<i>Panel</i>									
2B	0.77	79.49	98.99	0.76	79.49	98.99	0.76	81.08	98.89
3B	0.70	76.92	98.18	0.71	79.49	98.28	0.71	81.08	98.18
3ABC	0.68	64.10	99.09	0.72	66.67	99.19	0.70	70.27	99.19
3D	0.50	61.54	97.06	0.53	64.10	97.16	0.52	67.57	97.17
3CD	0.60	61.54	98.38	0.60	64.10	98.48	0.61	64.86	98.38
2C	0.10	64.10	76.82	0.08	56.41	76.52	0.09	56.76	76.46
<i>Field</i>									
2B	0.93	93.21	99.05	0.93	92.73	99.19	0.92	94.37	98.99
3B	0.92	95.06	98.10	0.92	93.94	98.37	0.91	96.25	98.28
3ABC	0.94	93.83	98.95	0.94	92.73	99.19	0.92	95.62	99.19
3D	0.74	73.46	96.96	0.74	72.73	97.26	0.73	75.00	97.27
3CD	0.75	69.13	98.29	0.74	68.48	98.58	0.74	70.00	98.48
2C	0.37	80.25	75.78	0.37	78.79	76.75	0.37	80.62	76.77

Supplementary Table S2. Assessment of the diagnostic performance for a range of ± 0.1 cut-off points of each of the NSP tests, for the detection of infection and/or carrier status in unvaccinated, vaccinated, published NSP panel, and known clinically infected field sera from cattle. Se = sensitivity (%); Sp = specificity (%), LR+ = likelihood ratio test for positive results; LR- = likelihood ratio test for negative results.

	Test	Cut-Off	Se	Sp	Classified	LR+	LR-
<i>Unvaccinated Infected Recovered</i>	Prionics	40	100.00	94.85	95.09	19.43	0.00
		45	100.00	98.08	98.17	52.16	0.00
		50	100.00	99.39	99.42	165.17	0.00
		55	100.00	99.60	99.61	247.75	0.00
		60	100.00	99.80	99.81	495.51	0.00
	IDvet Long	40	100.00	98.59	98.65	70.79	0.00
		45	100.00	98.69	98.75	76.23	0.00
		50	100.00	99.29	99.33	141.57	0.00
		55	100.00	99.50	99.52	198.20	0.00
		60	100.00	99.70	99.71	330.33	0.00
	IDvet Short	40	100.00	98.99	99.04	99.10	0.00
		45	100.00	99.19	99.23	123.87	0.00
		50	100.00	99.39	99.42	165.17	0.00
		55	100.00	99.50	99.52	198.20	0.00
		60	100.00	99.60	99.61	247.75	0.00
	2B	0.4	100.00	96.97	97.11	33.03	0
		0.45	100.00	98.08	98.17	52.16	0
		0.5	100.00	99.09	99.13	110.11	0
		0.55	95.74	99.60	99.42	237.21	0.04
		0.6	93.62	99.70	99.42	309.25	0.06
	3B	0.4	100.00	84.66	85.36	6.52	0.00
		0.45	100.00	92.53	92.87	13.39	0.00
		0.5	100.00	98.39	98.46	61.94	0.00
		0.55	97.87	98.79	98.75	80.83	0.02
		0.6	95.74	98.89	98.75	86.26	0.04
	3ABC	0.4	100.00	96.27	96.44	26.78	0.00
		0.45	100.00	98.18	98.27	55.06	0.00
		0.5	100.00	99.09	99.13	110.11	0.00
		0.55	93.62	99.19	98.94	115.97	0.06
		0.6	89.36	99.29	98.84	126.51	0.11
	3D	0.4	97.87	96.77	96.82	30.31	0.02
		0.45	97.87	96.97	97.01	32.33	0.02
		0.5	97.87	97.17	97.21	34.64	0.02
		0.55	97.87	97.88	97.88	46.19	0.02
		0.6	91.49	98.08	97.78	47.72	0.09
	3CD	0.4	97.87	98.08	98.07	51.05	0.02
		0.45	97.87	98.28	98.27	57.05	0.02
		0.5	97.87	98.49	98.46	64.66	0.02
		0.55	93.62	98.89	98.65	84.34	0.06
		0.6	91.49	99.29	98.94	129.52	0.09
	2C	0.4	87.23	65.29	66.28	2.51	0.20
		0.45	82.98	71.95	72.45	2.96	0.24
		0.5	82.98	76.69	76.97	3.56	0.22
		0.55	78.72	80.52	80.44	4.04	0.26
		0.6	76.60	83.96	83.62	4.77	0.28
<i>Unvaccinated Carrier</i>	Prionics	40	100.00	94.85	94.97	19.43	0.00
		45	100.00	98.08	98.12	52.16	0.00
		50	100.00	99.39	99.41	165.17	0.00
		55	100.00	99.60	99.61	247.75	0.00
		60	100.00	99.80	99.80	495.51	0.00

	IDvet Long	40	100.00	98.59	98.62	70.79	0.00
		45	100.00	98.69	98.72	76.23	0.00
		50	100.00	99.29	99.31	141.57	0.00
		55	100.00	99.50	99.51	198.20	0.00
		60	100.00	99.70	99.70	330.33	0.00
	IDvet Short	40	100.00	98.99	99.01	99.10	0.00
		45	100.00	99.19	99.21	123.87	0.00
		50	100.00	99.39	99.41	165.17	0.00
		55	100.00	99.50	99.51	198.20	0.00
		60	100.00	99.60	99.61	247.75	0.00
	2B	0.4	100.00	96.97	97.04	33.03	0.00
		0.45	100.00	98.59	98.62	70.79	0.00
		0.5	100.00	99.09	99.11	110.11	0.00
		0.55	90.91	99.60	99.41	225.23	0.09
		0.6	86.36	99.70	99.41	285.29	0.14
	3B	0.4	100.00	84.66	85.00	6.52	0.00
		0.45	100.00	92.53	92.69	13.39	0.00
		0.5	100.00	98.39	98.42	61.94	0.00
		0.55	100.00	98.79	98.82	82.58	0.00
		0.6	95.45	98.79	98.72	78.83	0.05
	3ABC	0.4	100.00	96.27	96.35	26.78	0.00
		0.45	100.00	98.18	98.22	55.06	0.00
		0.5	100.00	99.09	99.11	110.11	0.00
		0.55	90.91	99.19	99.01	112.61	0.09
		0.6	86.36	99.29	99.01	122.27	0.14
	3D	0.4	95.45	96.77	96.74	29.56	0.05
		0.45	95.45	96.97	96.94	31.53	0.05
		0.5	95.45	97.17	97.14	33.78	0.05
		0.55	95.45	97.88	97.83	45.05	0.05
		0.6	86.36	97.98	97.73	42.79	0.14
	3CD	0.4	95.45	98.08	98.03	49.79	0.05
		0.45	95.45	98.28	98.22	55.64	0.05
		0.5	95.45	98.49	98.42	63.06	0.05
		0.55	90.91	98.89	98.72	81.90	0.09
		0.6	90.91	99.19	99.01	112.61	0.09
	2C	0.4	77.27	65.29	65.55	2.23	0.35
		0.45	68.18	71.95	71.87	2.43	0.44
		0.5	68.18	76.69	76.51	2.93	0.41
		0.55	59.09	80.52	80.06	3.03	0.51
		0.6	59.09	83.96	83.42	3.68	0.49
<i>Vaccinated Infected Recovered</i>	Prionics	40	85.83	94.83	93.05	16.59	0.15
		45	84.21	98.11	95.37	44.54	0.16
		50	82.59	99.40	96.09	138.34	0.18
		55	82.19	99.80	96.33	412.99	0.18
		60	80.16	99.90	96.01	805.63	0.20
	IDvet Long	40	79.76	98.51	94.81	53.44	0.21
		45	76.92	98.61	94.33	55.22	0.23
		50	74.90	99.20	94.41	94.09	0.25
		55	72.87	99.40	94.17	122.06	0.27
		60	70.04	99.60	93.77	175.98	0.30
	IDvet Short	40	82.59	98.81	95.61	69.17	0.18
		45	80.16	99.10	95.37	89.51	0.20
		50	77.73	99.30	95.05	111.60	0.22
		55	74.90	99.40	94.57	125.46	0.25
		60	72.47	99.60	94.25	182.08	0.28
	2B	0.4	67.21	97.01	91.13	22.51	0.34
		0.45	63.56	98.01	91.21	31.94	0.37
		0.5	61.54	99.10	91.69	68.72	0.39
		0.55	55.87	99.40	90.81	93.58	0.44
		0.6	48.58	99.60	89.54	122.06	0.52
	3B	0.4	80.97	84.68	83.95	5.28	0.22
		0.45	77.73	92.54	89.62	10.42	0.24
		0.5	72.47	98.41	93.29	45.52	0.28
		0.55	70.45	98.81	93.21	59.00	0.30
		0.6	65.18	98.91	92.25	59.55	0.35

<i>Vaccinated Carrier</i>	3ABC	0.4	65.18	96.22	90.10	17.24	0.36
		0.45	62.75	98.21	91.21	35.04	0.38
		0.5	61.54	99.00	91.61	61.85	0.39
		0.55	57.09	99.20	90.89	71.71	0.43
		0.6	51.42	99.20	89.78	64.59	0.49
	3D	0.4	70.04	96.22	91.05	18.52	0.31
		0.45	68.83	96.52	91.05	19.76	0.32
		0.5	66.80	96.62	90.73	19.75	0.34
		0.55	62.75	97.51	90.65	25.23	0.38
		0.6	57.49	97.61	89.70	24.07	0.44
	3CD	0.4	61.54	97.51	90.42	24.74	0.39
		0.45	59.92	97.61	90.18	25.09	0.41
		0.5	57.89	98.11	90.18	30.62	0.43
		0.55	52.23	98.51	89.38	34.99	0.49
		0.6	49.80	98.81	89.14	41.71	0.51
	2C	0.4	55.06	65.47	63.42	1.59	0.69
		0.45	50.20	72.04	67.73	1.80	0.69
		0.5	44.13	76.92	70.45	1.91	0.73
		0.55	35.22	80.60	71.65	1.82	0.80
		0.6	30.36	83.98	73.40	1.90	0.83
	Prionics	40	92.86	94.85	94.59	18.04	0.08
		45	90.91	98.08	97.12	47.42	0.09
		50	90.26	99.39	98.17	149.08	0.10
		55	90.26	99.80	98.52	447.25	0.10
		60	88.31	99.90	98.34	875.12	0.12
	IDvet Long	40	90.26	98.59	97.47	63.89	0.10
		45	88.96	98.79	97.47	73.47	0.11
		50	86.36	99.29	97.55	122.27	0.14
		55	83.77	99.60	97.47	207.53	0.16
		60	80.52	99.80	97.21	398.98	0.20
	IDvet Short	40	88.96	98.99	97.64	88.16	0.11
		45	87.01	99.29	97.64	123.19	0.13
		50	83.77	99.50	97.38	166.03	0.16
		55	81.82	99.60	97.21	202.70	0.18
		60	77.92	99.70	96.77	257.40	0.22
	2B	0.4	75.32	97.07	94.15	25.74	0.25
		0.45	72.08	98.08	94.59	37.59	0.28
		0.5	70.13	99.19	95.28	86.87	0.30
		0.55	66.23	99.50	95.02	131.28	0.34
		0.6	57.79	99.70	94.06	190.91	0.42
	3B	0.4	83.77	84.66	84.54	5.46	0.19
		0.45	81.82	92.53	91.09	10.96	0.20
		0.5	75.97	98.39	95.37	47.06	0.24
		0.55	74.03	98.79	95.46	61.13	0.26
		0.6	66.23	98.89	94.50	59.67	0.34
	3ABC	0.4	73.38	96.27	93.19	19.65	0.28
		0.45	71.43	98.18	94.59	39.33	0.29
		0.5	70.78	99.09	95.28	77.94	0.29
		0.55	64.94	99.29	94.67	91.93	0.35
		0.6	59.09	99.29	93.89	83.66	0.41
	3D	0.4	63.64	96.77	92.31	19.71	0.38
		0.45	62.34	97.07	92.40	21.30	0.39
		0.5	61.04	97.17	92.31	21.60	0.40
		0.55	57.79	97.98	92.58	28.64	0.43
		0.6	53.90	98.08	92.14	28.11	0.47
	3CD	0.4	59.74	98.08	92.93	31.16	0.41
		0.45	59.09	98.28	93.01	34.45	0.42
		0.5	56.49	98.59	92.93	39.99	0.44
		0.55	49.35	98.99	92.31	48.91	0.51
		0.6	46.10	99.19	92.05	57.11	0.54
	2C	0.4	63.64	65.29	65.07	1.83	0.56
		0.45	57.14	71.95	69.96	2.04	0.60
		0.5	49.35	76.89	73.19	2.14	0.66
		0.55	40.26	80.52	75.11	2.07	0.74
		0.6	35.71	83.96	77.47	2.23	0.77

<i>Panel</i>	Prionics	40	94.44	94.85	94.84	18.35	0.06
		45	94.44	98.08	97.96	49.26	0.06
		50	91.67	99.39	99.12	151.40	0.08
		55	91.67	99.60	99.32	227.10	0.08
		60	86.11	99.80	99.32	426.69	0.14
	IDvet Long	40	100.00	98.59	98.64	70.79	0.00
		45	100.00	98.79	98.83	82.58	0.00
		50	91.67	99.29	99.03	129.77	0.08
		55	91.67	99.50	99.22	181.68	0.08
		60	86.11	99.80	99.32	426.69	0.14
	IDvet Short	40	94.44	98.99	98.83	93.59	0.06
		45	94.44	99.19	99.03	116.99	0.06
		50	91.67	99.50	99.22	181.68	0.08
		55	88.89	99.50	99.12	176.18	0.11
		60	86.11	99.60	99.12	213.34	0.14
	2B	0.4	97.22	96.97	96.98	32.12	0.03
		0.45	94.44	98.08	97.96	49.26	0.06
		0.5	94.44	99.09	98.93	103.99	0.06
		0.55	75.00	99.50	98.64	148.65	0.25
		0.6	69.44	99.70	98.64	229.40	0.31
	3B	0.4	94.44	84.66	85.00	6.16	0.07
		0.45	94.44	92.53	92.60	12.65	0.06
		0.5	88.89	98.39	98.05	55.06	0.11
		0.55	88.89	98.79	98.44	73.41	0.11
		0.6	86.11	98.79	98.34	71.11	0.14
	3ABC	0.4	86.11	96.27	95.91	23.06	0.14
		0.45	75.00	98.18	97.37	41.29	0.25
		0.5	75.00	99.19	98.34	92.91	0.25
		0.55	69.44	99.29	98.25	98.31	0.31
		0.6	66.67	99.29	98.15	94.38	0.34
	3D	0.4	80.56	96.87	96.30	25.75	0.20
		0.45	72.22	96.97	96.11	23.86	0.29
		0.5	72.22	97.17	96.30	25.56	0.29
		0.55	69.44	97.88	96.88	32.77	0.31
		0.6	66.67	97.98	96.88	33.03	0.34
	3CD	0.4	75.00	98.08	97.27	39.12	0.25
		0.45	75.00	98.28	97.47	43.72	0.25
		0.5	72.22	98.49	97.57	47.71	0.28
		0.55	58.33	98.89	97.47	52.55	0.42
		0.6	50.00	99.29	97.57	70.79	0.50
	2C	0.4	72.22	65.29	65.53	2.08	0.43
		0.45	72.22	71.95	71.96	2.57	0.39
		0.5	69.44	76.69	76.44	2.98	0.40
		0.55	63.89	80.52	79.94	3.28	0.45
		0.6	61.11	83.96	83.15	3.81	0.46
<i>Field</i>	Prionics	40	97.48	94.85	95.22	18.94	0.03
		45	97.48	98.08	98.00	50.85	0.03
		50	96.86	99.39	99.04	159.97	0.03
		55	96.86	99.60	99.22	239.96	0.03
		60	96.86	99.80	99.39	479.93	0.03
	IDvet Long	40	100.00	98.59	98.78	70.79	0.00
		45	100.00	98.69	98.87	76.23	0.00
		50	100.00	99.29	99.39	141.57	0.00
		55	100.00	99.50	99.57	198.20	0.00
		60	100.00	99.70	99.74	330.33	0.00
	IDvet Short	40	98.11	98.99	98.87	97.23	0.02
		45	98.11	99.29	99.13	138.90	0.02
		50	97.48	99.39	99.13	161.01	0.03
		55	97.48	99.50	99.22	193.21	0.03
		60	96.86	99.60	99.22	239.96	0.03
	2B	0.4	98.11	96.97	97.13	32.41	0.02
		0.45	96.23	98.08	97.83	50.19	0.04
		0.5	96.23	99.09	98.70	105.96	0.04
		0.55	93.71	99.50	98.70	185.74	0.06
		0.6	91.82	99.70	98.61	303.32	0.08

3B	0.4	98.11	84.66	86.52	6.40	0.02
	0.45	97.48	92.53	93.22	13.06	0.03
	0.5	97.48	98.39	98.26	60.38	0.03
	0.55	96.86	98.69	98.43	73.83	0.03
	0.6	94.97	98.89	98.35	85.56	0.05
3ABC	0.4	96.86	96.27	96.35	25.94	0.03
	0.45	96.23	98.18	97.91	52.98	0.04
	0.5	96.23	99.09	98.70	105.96	0.04
	0.55	96.23	99.19	98.78	119.20	0.04
	0.6	96.23	99.29	98.87	136.23	0.04
3D	0.4	77.36	96.77	94.09	23.96	0.23
	0.45	76.73	97.07	94.26	26.22	0.24
	0.5	75.47	97.17	94.17	26.71	0.25
	0.55	69.18	97.88	93.91	32.65	0.31
	0.6	63.52	98.08	93.30	33.13	0.37
3CD	0.4	76.73	98.08	95.13	40.02	0.24
	0.45	73.58	98.39	94.96	45.58	0.27
	0.5	71.07	98.49	94.70	46.95	0.29
	0.55	58.49	98.99	93.39	57.96	0.42
	0.6	50.31	99.29	92.52	71.23	0.50
2C	0.4	88.68	65.29	68.52	2.55	0.17
	0.45	84.28	71.95	73.65	3.00	0.22
	0.5	81.76	76.69	77.39	3.51	0.24
	0.55	76.73	80.52	80.00	3.94	0.29
	0.6	74.21	83.96	82.61	4.63	0.31