Inter-laboratory collaborative trial of real-time PCR method phase 1: Appendices

Appendix 1 Table A1

Spectrophotometer (NanoDropTM 1000) data for beef, horse and pork in raw and processed formats across the various % w/w combinations. The horse and pork % w/w/ combinations were in a matrix of beef. The data relate to individual extracts prior to pooling, absorbance at 260 and 280 nm.

Sample matrix	A260	A280	A260/A280	A260/A230	ng/µL
100% raw Beef extract 1	3.23	1.60	2.02	1.98	161.33
100% raw Beef extract 2	3.15	1.54	2.04	2.06	157.32
100% raw Beef extract 3	2.87	1.42	2.03	1.97	143.70
100% raw Horse extract 1	2.21	1.12	1.98	1.99	110.69
100% raw Horse extract 2	2.75	1.39	1.97	2.06	137.32
100% raw Horse extract 3	2.24	1.13	1.98	1.77	112.02
100% raw Pork extract 1	4.8	2.39	2.01	2.03	240.08
100% raw Pork extract 2	4.6	2.28	2.02	2.13	230.25
100% raw Pork extract 3	4.31	2.16	2.00	2.00	215.73
10% raw Pork extract 1	3.68	1.88	1.96	2.24	183.89
10% raw Pork extract 2	3.44	1.74	1.98	2.24	172.04
10% raw Pork extract 3	3.12	1.58	1.98	2.22	156.01
3% raw Pork extract 1	3.41	1.74	1.96	2.17	170.25

Sample matrix	A260	A280	A260/A280	A260/A230	ng/µL
3% raw Pork extract 2	4.35	2.21	1.97	2.19	217.42
3% raw Pork extract 3	3.37	1.71	1.97	2.19	168.72
1% raw Pork extract 1	2.51	1.27	1.97	2.13	125.53
1% raw Pork extract 2	1.93	0.97	2	2.03	96.68
1% raw Pork extract 3	2.04	1.02	2.01	2.18	102.10
0.5% raw Pork extract 1	4.59	2.37	1.93	1.87	229.28
0.5% raw Pork extract 2	4.4	2.24	1.97	2.19	220.11
0.5% raw Pork extract 3	3.29	1.68	1.95	2.12	164.40
0.1% raw Pork extract 1	3.15	1.63	1.93	1.83	157.40
0.1% raw Pork extract 2	2.82	1.43	1.97	2.13	140.83
0.1% raw Pork extract 3	2.63	1.34	1.96	2.05	131.30
10% processed Pork extract 1	1.25	0.71	1.76	0.96	62.40
10% processed Pork extract 2	0.81	0.39	2.05	1.96	40.45
10% processed Pork extract 3	0.76	0.38	2.01	1.9	38.15
3% processed Pork extract 1	0.87	0.46	1.91	1.48	43.67
3% processed Pork extract 2	0.91	0.47	1.92	1.65	45.37
3% processed Pork extract 3	0.86	0.44	1.97	1.88	43.19
1% processed Pork extract 1	0.93	0.48	1.94	2.36	46.32

Sample matrix	A260	A280	A260/A280	A260/A230	ng/µL
1% processed Pork extract 2	0.9	0.47	1.91	2.09	45.24
1% processed Pork extract 3	0.84	0.44	1.9	2.32	42.21
0.5% processed Pork extract 1	1.29	0.68	1.91	2.22	64.73
0.5% processed Pork extract 2	1.23	0.65	1.91	2.04	61.73
0.5% processed Pork extract 3	0.94	0.49	1.92	1.95	47.14
0.1% processed Pork extract 1	1.33	0.72	1.83	1.51	66.25
0.1% processed Pork extract 2	1.1	0.59	1.86	1.71	54.93
0.1% processed Pork extract 3	1.32	0.7	1.9	2.1	66.23
10% raw Horse extract 1	4	2.07	1.93	2.14	199.83
10% raw Horse extract 2	3.7	1.91	1.93	2.15	185.10
10% raw Horse extract 3	3.97	2.04	1.95	2.16	198.31
3% raw Horse extract 1	3.92	2.03	1.93	2.16	195.80
3% raw Horse extract 2	2.95	1.52	1.93	2.1	147.43
3% raw Horse extract 3	4.16	2.14	1.94	2.18	208.20
1% raw Horse extract 1	3.12	1.61	1.94	1.93	156.18
1% raw Horse extract 2	3.56	1.81	1.97	2.2	178.06
1% raw Horse extract 3	3.16	1.64	1.93	1.81	158.20

Sample matrix	A260	A280	A260/A280	A260/A230	ng/µL
0.5% raw Horse extract 1	3.97	2.04	1.95	2.06	198.32
0.5% raw Horse extract 2	3.34	1.71	1.95	2.11	166.86
0.5% raw Horse extract 3	3.36	1.72	1.96	2.23	167.92
0.1% raw Horse extract 1	4.56	2.34	1.95	2.19	227.99
0.1% raw Horse extract 2	3.3	1.7	1.95	2.21	165.07
0.1% raw Horse extract 3	4.49	2.32	1.94	2	224.74
10% processed horse extract 1	1.19	0.62	1.93	1.81	59.40
10% processed horse extract 2	1.27	0.66	1.93	1.88	63.37
10% processed horse extract 3	1.35	0.71	1.91	1.73	67.49
3% processed horse extract 1	0.94	0.49	1.91	1.73	47.22
3% processed horse extract 2	1.05	0.53	1.98	1.93	52.35
3% processed horse extract 3	1.23	0.62	1.98	1.93	61.67
1% processed horse extract 1	1.29	0.76	1.7	0.79	64.41
1% processed horse extract 2	1.58	0.93	1.69	0.81	79.08
1% processed horse extract 3	1.45	0.86	1.68	0.77	72.51

Sample matrix	A260	A280	A260/A280	A260/A230	ng/µL
0.5% processed horse extract 1	1.53	0.84	1.81	1.78	76.58
0.5% processed horse extract 2	1.54	0.84	1.84	1.91	77.05
0.5% processed horse extract 3	0.9	0.5	1.81	1.78	45.24
0.1% processed horse extract 1	1.43	0.77	1.85	2.21	71.45
0.1% processed horse extract 2	1.53	0.83	1.85	2.24	76.48
0.1% processed horse extract 3	1.53	0.83	1.84	2.22	76.73

Appendix II: Authentication of meat species by Sanger sequencing, alignment figures

Figure A1: Alignment figure for pork forward and reverse cytochrome B sequences against the pork reference sequence (NC_000845).
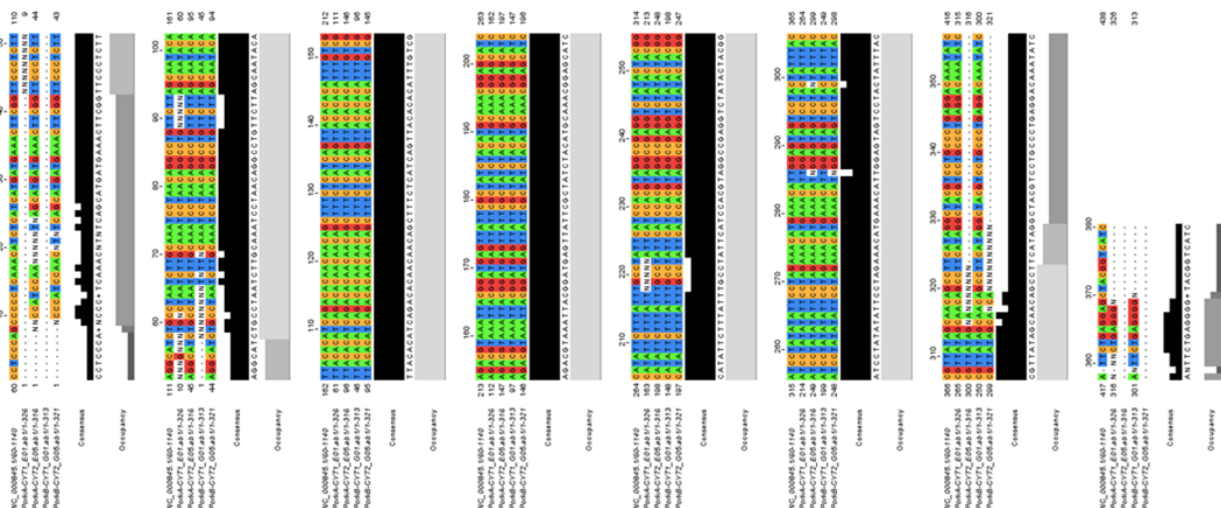

Figure A2: Alignment figure for horse forward and reverse cytochrome B sequences against the horse reference sequence (D82932).
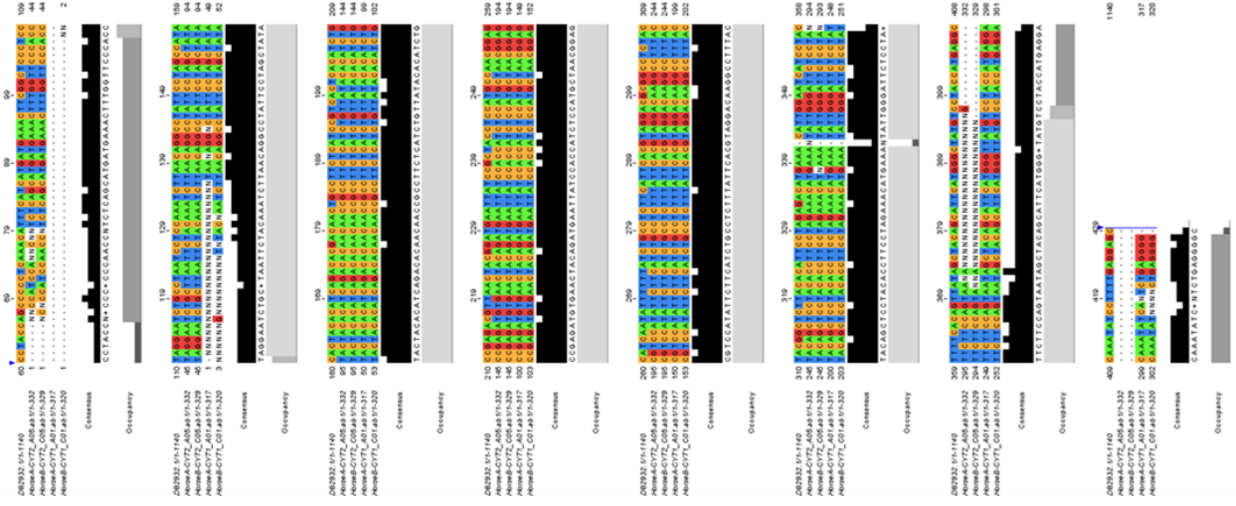
Figure A3: Alignment figure for beef forward and reverse cytochrome B sequences against the beef reference sequence (D34635).