Inter-laboratory collaborative trial of real-time PCR method phase 2: Appendix 2 Collaborative trial raw data

Collaborative trial MA0201 practical instructions Appendix 1

Table A1: Sequence of the primers and probe for each of the horse, pork and mammalian (myostatin) assays used in the collaborative trial.

Target	Assay ref	Sequence (5'-3')	Labelling
Horse (Köppel, R. et al. 2011)	Forward	CCAACTTCATCATGGACAACGC	-
Horse (Köppel, R. et al. 2011)	Reverse	GTTAAAGCTTGGCTCGACACG	-
Horse (Köppel, R. et al. 2011)	Probe	AAGTGCATCCCCGTGGCCCCTCA	6 FAM NFQ
Pork (Köppel, R. et al. 2011)	Forward	GGAGTGTGTATCCCGTAGGTG	-
Pork (Köppel, R. et al. 2011)	Reverse	CTGGGGACATGCAGAGAGTG	-
Pork (Köppel, R. et al. 2011)	Probe	TCTGACGTGACTCCCCGACCTGG	6 FAM NFQ

Target	Assay ref	Sequence (5'-3')	Labelling
Mammalian Myostatin (Laube, I. et al. 2013)	Forward	TCTGACGTGACTCCCCGACCTGG	-
Mammalian Myostatin (Laube, I. et al. 2013)	Reverse	ATACCAGTGCCTGGGTTCAT	-
Mammalian Myostatin (Laube, I. et al. 2013)	Probe	CCCATGAAAGACGGTACAAGGTATACTG	6 FAM NFQ

Köppel, R., Ruf, J., Rentsch, J. Multiplex real-time PCR for the detection and quantification of DNA from beef, pork, horse and sheep. Eur Food Res Technol. 2011;232(1):151-5. 10.1007/s00217-010-1371-y

Laube, I., Spiegelberg, A., Butschke, A., Zagon, J., Schauzu, M., Kroh, L., et al. Methods for the detection of beef and pork in foods using real-time polymerase chain reaction. International Journal Of Food Science & Technology. 2003;38(2):111-8. 10.1046/j.1365-2621.2003.00651.x.

Collaborative trial MA0201 practical instructions Appendix 2

Suggested plate plans for the horse assay and the mammalian myostatin assay.

Plate 1 Horse

Letter	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1/H	Std 1/H	Std 1/H	Std 2/H	Std 2/H	Std 2/H	Std 3/H	Std 3/H	Std 3/H	Std 4/H	Std 4/H	Std 4/H
B	Std 5/H	Std 5/H	Std 5/H	NTC	NTC	NTC	1	1	1	2	2	2
C	3	3	3	4	4	4	5	5	5	6	6	6
D	7	7	7	8	8	8	9	9	9	10	10	10

Letter	1	2	3	4	5	6	7	8	9	10	11	12
E	Std 1/H	Std 1/H	Std 1/H	Std 2/H	Std 2/H	Std 2/H	Std 3/H	Std 3/H	Std 3/H	Std 4/H	Std 4/H	Std 4/H
F	Std 5/H	Std 5/H	Std 5/H	NTC	NTC	NTC	1	1	1	2	2	2
G	3	3	3	4	4	4	5	5	5	6	6	6
H	7	7	7	8	8	8	9	9	9	10	10	10

Plate 2 Horse

Letter	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1/H	Std 1/H	Std 1/H	Std 2/H	Std 2/H	Std 2/H	Std 3/H	Std 3/H	Std 3/H	Std 4/H	Std 4/H	Std 4/H
B	Std 5/H	Std 5/H	Std 5/H	NTC	NTC	NTC	11	11	11	12	12	12
C	13	13	13	14	14	14	15	15	15	16	16	16
D	17	17	17	18	18	18	19	19	19	20	20	20
E	Std 1/H	Std 1/H	Std 1/H	Std 2/H	Std 2/H	Std 2/H	Std 3/H	Std 3/H	Std 3/H	Std 4/H	Std 4/H	Std 4/H
F	Std 5/H	Std 5/H	Std 5/H	NTC	NTC	NTC	11	11	11	12	12	12
G	13	13	13	14	14	14	15	15	15	16	16	16
H	17	17	17	18	18	18	19	19	19	20	20	20

Plate 3 Horse

Letter	1	2	3	4	5	6	7	8	9	1
A	Std 1/H	Std 1/H	Std 1/H	Std 2/H	Std 2/H	Std 2/H	Std 3/H	Std 3/H	Std 3/H	S 4
B	Std 5/H	Std 5/H	Std 5/H	NTC	NTC	NTC	21	21	21	2
C	23	23	23	24	24	24	25	25	25	2
D	27	27	27	28	28	28	29	29	29	3
E	Std 1/H	Std 1/H	Std 1/H	Std 2/H	Std 2/H	Std 2/H	Std 3/H	Std 3/H	Std 3/H	S 4
F	Std 5/H	Std 5/H	Std 5/H	NTC	NTC	NTC	21	21	21	2
G	23	23	23	24	24	24	25	25	25	2
H	27	27	27	28	28	28	29	29	29	3

Suggested plate plans for the pork assay and the mammalian myostatin assay

Plate 1 Pork

Letter	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1/P	Std 1/P	Std 1/P	Std 2/P	Std 2/P	Std 2/P	Std 3/P	Std 3/P	Std 3/P	Std 4/P	Std 4/P	Std 4/P
B	Std 5/P	Std 5/P	Std 5/P	NTC	NTC	NTC	1	1	1	2	2	2
C	3	3	3	4	4	4	5	5	5	6	6	6
D	7	7	7	8	8	8	9	9	9	10	10	10
E	Std 1/P	Std 1/P	Std 1/P	Std 2/P	Std 2/P	Std 2/P	Std 3/P	Std 3/P	Std 3/P	Std 4/P	Std 4/P	Std 4/P

Letter	1	2	3	4	5	6	7	8	9	10	11	12
F	Std 5/P	Std 5/P	Std 5/P	NTC	NTC	NTC	1	1	1	2	2	2
G	3	3	3	4	4	4	5	5	5	6	6	6
H	7	7	7	8	8	8	9	9	9	10	10	10

Plate 2 Pork

Letter	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1/P	Std 1/P	Std 1/P	Std 2/P	Std 2/P	Std 2/P	Std 3/P	Std 3/P	Std 3/P	Std 4/P	Std 4/P	Std 4/P
B	Std 5/P	Std 5/P	Std 5/P	NTC	NTC	NTC	11	11	11	12	12	12
C	13	13	13	14	14	14	15	15	15	16	16	16
D	17	17	17	18	18	18	19	19	19	20	20	20
E	Std 1/P	Std 1/P	Std 1/P	Std 2/P	Std 2/P	Std 2/P	Std 3/P	Std 3/P	Std 3/P	Std 4/P	Std 4/P	Std 4/P
F	Std 5/P	Std 5/P	Std 5/P	NTC	NTC	NTC	11	11	11	12	12	12
G	13	13	13	14	14	14	15	15	15	16	16	16
H	17	17	17	18	18	18	19	19	19	20	20	20

Plate 3 Pork

Letter	1	2	3	4	5	6	7	8	9	1

A	Std 1/P	Std 1/P	Std 1/P	Std 2/P	Std 2/P	Std 2/P	Std 3/P	Std 3/P	Std 3/P	S 4
B	Std 5/P	Std 5/P	Std 5/P	NTC	NTC	NTC	21	21	21	2
C	23	23	23	24	24	24	25	25	25	2
D	27	27	27	28	28	28	29	29	29	3
E	Std 1/P	Std 1/P	Std 1/P	Std 2/P	Std 2/P	Std 2/P	Std 3/P	Std 3/P	Std 3/P	S 4
F	Std 5/P	Std 5/P	Std 5/P	NTC	NTC	NTC	21	21	21	2
G	23	23	23	24	24	24	25	25	25	2
H	27	27	27	28	28	28	29	29	29	3

Appendix 2: Collaborative trial raw data

Horse in processed beef

Nominal level (%)	**0.1**	**0.1**	**0.5**	**0.5**	**1**	**1**	**3**	**3**
Sample ID	PCR_17	PCR_18	PCR_09	PCR_12	PCR_06	PCR_02	PCR086	PC
Lab no.1	0.26	0.2	1.04	0.83	2.34	2.58	4.55	5.1
Lab no.2	0.23	0.25	1.17	1.15	2.2	2.22	3.92	3.9
Lab no.3	0.19	0.17	1.01	0.8	2.05	1.85	3.72	3.4

Nominal level(%)	0.1	0.1	0.5	0.5	1	1	3	3
Lab no.4	0.24	0.33	0.89	1.04	1.76	1.81	3.65	3.6
Lab no.5	0.15	0.18	0.74	0.79	1.62	1.57	3.07	3.1
Lab no.6	0.16	0.16	0.9	0.81	1.76	1.72	3.5	3.5
Lab no.7	0.13	0.16	0.8	0.68	1.63	1.47	3.2	3.3
Lab no.8	0.16	0.18	0.72	0.88	1.59	1.4	3.05	2.9
Lab no.9	0.14	0.12	0.77	0.78	1.52	1.61	2.84	3.1
Lab no.10	0.1	0.1	0.5	0.5	1	1	3	3
Lab no.11	0.1	0.18	0.87	0.77	1.83	1.9	3.47	3.4
Lab no.12	0.17	0.23	1.66 c	1.14 c	3.38 g	3.5 g	5.45 g	5.2
Lab no.13	0.09	0.14	ND	0.69	1.67	1.85	3.47	3.0
Lab no.14	0.1	0.13	0.62	0.68	1.17	1.58	2.42	3.1
Lab no.15	0.21	0.3	0.98	ND	1.86	1.73	5.07 c	3.8

Pork in processed beef

Nominal level (%)	0.1	0.1	0.5	0.5	1	1	3	3
Sample ID	PCR_13	PCR_14	PCR_03	PCR_11	PCR_07	PCR_21	PCR_25	PC
Lab no.1	0.11	0.09	0.6	0.6	1.28	1.66	3.31	2.7
Lab no.2	0.18	0.13	0.84	0.76	1.74	1.58	2.67	2.6
Lab no.3	0.18	0.17	0.57	0.64	1.21	1.14	2.85 c	1.8
Lab no.4	0.19	0.22	0.72	0.88	1.89	1.49	2.37	2.4
Lab no.5	0.09	0.12	0.5	0.45	1.36	1.37	2.41	2.3
Lab no.6	0.1	0.11	0.58	0.55	1.19	1.57	2.16	2.4
Lab no.7	0.09	0.16	0.63	0.61	1.3	1.36	2.29	1.9
Lab no.8	0.19	0.2	0.71	0.7	1.36	1.42	2.54	2.5
Lab no.9	0.18	0.15	0.61	0.75	1.31	1.47	2.69	2.4
Lab no.10	0.1	0.1	0.5	0.5	0.5	1	3	3
Lab no.11	0.13	0.17	0.6	0.77	1.33	1.56	2.48	2.5
Lab no.12	0.44 g	0.48 g	1.38 g	1.55 g	2.7 g	3.89 g	7.39 g	5.3

Nominal level(%)	0.1	0.1	0.5	0.5	1	1	3	3
Lab no.13	0.11	0.1	0.54	0.42	1	1.3	2.17	2.3
Lab no.14	0.09	0.07	0.41	0.53	0.93	0.9	1.3	1.6
Lab no.15	0.1	0.09	0.74	0.56	2.62	1.9	6.26 c	2.2

Pork in raw beef

Nominal level (%)	0.1	0.1	0.5	0.5	1	1	3	3
Sample ID	PCR_23	PCR_28	PCR_30	PCR_04	PCR_27	PCR_01	PCR_16	PC
Lab no.1	0.13	0.12	0.57	0.48	1.59	1.26	3.17	3.0
Lab no.2	0.18	0.14	0.74	0.87	1.59	1.75	3.53	3.9
Lab no.3	0.07	0.05	0.63	0.43	1.63	0.99	3.83	2.6
Lab no.4	0.07	0.16	0.64	0.82	1.22	1.72	4.27	4.6
Lab no.5	0.13	0.1	0.64	0.42	1.29	1.09	2.78	3.0
Lab no.6	0.12	0.08	0.57	0.55	1.18	1.03	2.91	2.7
Lab no.7	0.07	0.11	0.59	0.62	0.99	1.48	3.88 c	1.8

Nominal level(%)	0.1	0.1	0.5	0.5	1	1	3	3
Lab no.8	0.14	0.13	0.74	0.55	1.37	1.42	3.49	3.5
Lab no.9	0.18	0.13	0.61	0.57	1.32	1.15	3.34	3.0
Lab no.10	0.1	0.1	1	0.5	1	1	3	3
Lab no.11	0.1	0.12	0.65	0.57	1.19	1.18	3.31	3.3
Lab no.12	0.66 g	0.48 g	1.77 g	1.29 g	3.08 g	2.77	6.15 g	4.8
Lab no.13	0.08	0.09	0.49	0.5	1.1	1.11	2.56	2.6
Lab no.14	0.03	0.05	0.33	0.51	0.58	1.01	2.57	2.5
Lab no.15	0.09	0.08	0.88	0.85	1.83	1.26	3.35 c	7.8

Calibration performance reported by laboratories

Lab number	Pork 1	Pork 2	Pork 3	Horse 1	Horse 2	Horse 3
1	0.999	0.999	0.994	0.998	0.997	0.999
2	0.993	0.995	0.994	0.996	0.997	0.998
3	0.997	0.985	0.998	0.994	0.996	0.994
4	0.994	0.996	0.997	0.990	0.997	0.999
5	0.998	0.998	0.999	0.992	0.998	0.995

Lab number	Pork 1	Pork 2	Pork 3	Horse 1	Horse 2	Horse 3
6	0.998	0.998	0.995	0.998	0998	0.992
7	0.998	0.997	0.998	0.999	0.996	0.999
8	0.996	0.997	0.999	0.995	0.991	0.998
9	0.999	0.999	0.999	0.995	0.993	0.953#
10	0.991	0.993	0.997	0.990	0.996	0.994
11	0.999	0.998	0.998	0.999	0.998	0.991
12	0.996	0.999	0.997	0.999	1.00	0.995
13	0.999	0.995	0.999	0.995	0.993	0.998
14	0.998	0.999	0.995	0.977#	0.996	0.988
15	0.991	0.997	0.963#	0.977#	0.958#	0.993

Acceptance criterion _>0.98, # indicates <0.98. Assay plate number indicated by 1, 2 or 3 following the target matrix of pork or horse.

PCR efficiency reported by laboratories (PCR efficiency %)

Lab number	Pork 1	Pork 2	Pork 3	Horse 1	Horse 2	Horse 3
1	96.6	92.5	91.5	101	103	96.4
2	83.2#	89.3	84.9#	87.7	86.6	88.9
3	99.1	91.0	97.2	89.7	94.5	87.6
4	91.6	88.4	94.7	95.0	85.4	88.1
5	98.5	100	93.3	97.0	93.0	95.2

Lab number	Pork 1	Pork 2	Pork 3	Horse 1	Horse 2	Horse 3
6	100	98.8	93.2	91.6	96.8	91.3
7	90.2	91.6	96.2	94.6	94.4	96.9
8	95.8	100	93.3	97.0	93.0	95.2
9	94.8	89.8	90.6	93.7	93.7	98.3
10	94.8	90.5	90.7	104	93.3	89.8
11	93.8	95.6	90.9	94.2	97.3	91.0
12	80.5#	79.5#	71.8#	72.1#	82.8#	89.1
13	95.6	98.9	96.1	92.9	102	98.0
14	95.4	99.1	104	92.4	95.9	88.8
15	106	105	105	104	109	108

Acceptance criterion 85 to 115%, # indicates value outside acceptance criterion. Assay plate number indicated by 1, 2 or 3 following the target matrix of pork or horse.