

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 | GTAAAGCTTGCTCGACACG | - |
| 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 |
| Mammalian Myostatin (Laube, I. et al. 2013) | Forward | TCTGACGTGACTCCCCGACCTGG | - |
| Mammalian Myostatin (Laube, I. et al. 2013) | Reverse | ATACCAGTGCCTGGGTTTCAT | - |
| Mammalian Myostatin (Laube, I. et al. 2013) | Probe | CCCATGAAAGACGGTACAAGGTATAC TG | 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 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-------------|
| 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 | Horse assay |
| 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 | - |

| Letter | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-----------|
| D | 7 | 7 | 7 | 8 | 8 | 8 | 9 | 9 | 9 | 10 | 10 | 10 | - |
| 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 | Myostatin |
| 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 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-------------|
| 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 | Horse assay |
| 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 | Myostatin |
| 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 | 10 | 11 | 12 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-------------|
| 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 | Horse assay |
| B | Std 5/H | Std 5/H | Std 5/H | NTC | NTC | NTC | 21 | 21 | 21 | 22 | 22 | 22 | - |
| C | 23 | 23 | 23 | 24 | 24 | 24 | 25 | 25 | 25 | 26 | 26 | 26 | - |
| D | 27 | 27 | 27 | 28 | 28 | 28 | 29 | 29 | 29 | 30 | 30 | 30 | - |
| 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 | Myostatin |
| F | Std 5/H | Std 5/H | Std 5/H | NTC | NTC | NTC | 21 | 21 | 21 | 22 | 22 | 22 | - |
| G | 23 | 23 | 23 | 24 | 24 | 24 | 25 | 25 | 25 | 26 | 26 | 26 | - |
| H | 27 | 27 | 27 | 28 | 28 | 28 | 29 | 29 | 29 | 30 | 30 | 30 | - |

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 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------|
| 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 | Pork assay |
| 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 | Myostatin |
| 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 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------|
| 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 | Pork assay |
| 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 | Myostatin |
| 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 | 10 | 11 | 12 | Note |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------|
| 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 | Pork assay |
| B | Std 5/P | Std 5/P | Std 5/P | NTC | NTC | NTC | 21 | 21 | 21 | 22 | 22 | 22 | - |
| C | 23 | 23 | 23 | 24 | 24 | 24 | 25 | 25 | 25 | 26 | 26 | 26 | - |
| D | 27 | 27 | 27 | 28 | 28 | 28 | 29 | 29 | 29 | 30 | 30 | 30 | - |
| 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 | Myostatin |
| F | Std 5/P | Std 5/P | Std 5/P | NTC | NTC | NTC | 21 | 21 | 21 | 22 | 22 | 22 | - |
| G | 23 | 23 | 23 | 24 | 24 | 24 | 25 | 25 | 25 | 26 | 26 | 26 | - |
| H | 27 | 27 | 27 | 28 | 28 | 28 | 29 | 29 | 29 | 30 | 30 | 30 | - |

Appendix 2: Collaborative trial raw data

Horse in processed beef

| Nominal level (%) | 0.1 | 0.1 | 0.5 | 0.5 | 1 | 1 | 3 | 3 | 10 | 10 |
|-------------------|--------|--------|--------|--------|--------|--------|--------|--------|---------|--------|
| Sample ID | PCR_17 | PCR_18 | PCR_09 | PCR_12 | PCR_06 | PCR_02 | PCR086 | PCR_05 | PCR_19 | PCR_26 |
| Lab no.1 | 0.26 | 0.2 | 1.04 | 0.83 | 2.34 | 2.58 | 4.55 | 5.11 c | 13.44 | 10.65 |
| Lab no.2 | 0.23 | 0.25 | 1.17 | 1.15 | 2.2 | 2.22 | 3.92 | 3.9 | 12.83 | 10.15 |
| Lab no.3 | 0.19 | 0.17 | 1.01 | 0.8 | 2.05 | 1.85 | 3.72 | 3.47 | 10.96 | 11.26 |
| Lab no.4 | 0.24 | 0.33 | 0.89 | 1.04 | 1.76 | 1.81 | 3.65 | 3.67 | 13.18 | 11.63 |
| Lab no.5 | 0.15 | 0.18 | 0.74 | 0.79 | 1.62 | 1.57 | 3.07 | 3.19 | 11.18 | 10.26 |
| Lab no.6 | 0.16 | 0.16 | 0.9 | 0.81 | 1.76 | 1.72 | 3.5 | 3.55 | 10.06 | 9.74 |
| Lab no.7 | 0.13 | 0.16 | 0.8 | 0.68 | 1.63 | 1.47 | 3.2 | 3.35 | 9.57 | 10.98 |
| Lab no.8 | 0.16 | 0.18 | 0.72 | 0.88 | 1.59 | 1.4 | 3.05 | 2.99 | 9.16 | 10.73 |
| Lab no.9 | 0.14 | 0.12 | 0.77 | 0.78 | 1.52 | 1.61 | 2.84 | 3.1 | 9.18 | 10.62 |
| Lab no.10 | 0.1 | 0.1 | 0.5 | 0.5 | 1 | 1 | 3 | 3 | 10 | 10 |
| Lab no.11 | 0.1 | 0.18 | 0.87 | 0.77 | 1.83 | 1.9 | 3.47 | 3.42 | 10.95 | 10.12 |
| Lab no.12 | 0.17 | 0.23 | 1.66 c | 1.14 c | 3.38 g | 3.5 g | 5.45 g | 5.29 g | 9.98 | 12.08 |
| Lab no.13 | 0.09 | 0.14 | ND | 0.69 | 1.67 | 1.85 | 3.47 | 3.02 | 8.99 | 9.08 |
| Lab no.14 | 0.1 | 0.13 | 0.62 | 0.68 | 1.17 | 1.58 | 2.42 | 3.12 | 9.08 | 7.94 |
| Lab no.15 | 0.21 | 0.3 | 0.98 | ND | 1.86 | 1.73 | 5.07 c | 3.86 c | 18.68 g | 20 g |

Pork in processed beef

| Nominal level (%) | 0.1 | 0.1 | 0.5 | 0.5 | 1 | 1 | 3 | 3 | 10 | 10 |
|-------------------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| Sample ID | PCR_13 | PCR_14 | PCR_03 | PCR_11 | PCR_07 | PCR_21 | PCR_25 | PCR_22 | PCR_20 | PCR_24 |
| Lab no.1 | 0.11 | 0.09 | 0.6 | 0.6 | 1.28 | 1.66 | 3.31 | 2.78 | 5.85 | 7.48 |
| Lab no.2 | 0.18 | 0.13 | 0.84 | 0.76 | 1.74 | 1.58 | 2.67 | 2.6 | 7.28 | 7.04 |
| Lab no.3 | 0.18 | 0.17 | 0.57 | 0.64 | 1.21 | 1.14 | 2.85 c | 1.86 c | 9.24 | 7.42 |
| Lab no.4 | 0.19 | 0.22 | 0.72 | 0.88 | 1.89 | 1.49 | 2.37 | 2.42 | 9.16 | 6.82 |
| Lab no.5 | 0.09 | 0.12 | 0.5 | 0.45 | 1.36 | 1.37 | 2.41 | 2.34 | 7.63 | 7.24 |
| Lab no.6 | 0.1 | 0.11 | 0.58 | 0.55 | 1.19 | 1.57 | 2.16 | 2.45 | 6.58 | 6.56 |
| Lab no.7 | 0.09 | 0.16 | 0.63 | 0.61 | 1.3 | 1.36 | 2.29 | 1.99 | 8 | 5.8 |
| Lab no.8 | 0.19 | 0.2 | 0.71 | 0.7 | 1.36 | 1.42 | 2.54 | 2.55 | 7.24 | 7.29 |
| Lab no.9 | 0.18 | 0.15 | 0.61 | 0.75 | 1.31 | 1.47 | 2.69 | 2.41 | 6.62 | 6.78 |
| Lab no.10 | 0.1 | 0.1 | 0.5 | 0.5 | 0.5 | 1 | 3 | 3 | 10 | 10 |
| Lab no.11 | 0.13 | 0.17 | 0.6 | 0.77 | 1.33 | 1.56 | 2.48 | 2.52 | 6.82 | 7.09 |
| Lab no.12 | 0.44 g | 0.48 g | 1.38 g | 1.55 g | 2.7 g | 3.89 g | 7.39 g | 5.38 g | 12.14 g | 12.49 g |
| Lab no.13 | 0.11 | 0.1 | 0.54 | 0.42 | 1 | 1.3 | 2.17 | 2.33 | 6.41 | 6.13 |
| Lab no.14 | 0.09 | 0.07 | 0.41 | 0.53 | 0.93 | 0.9 | 1.3 | 1.64 | 6.19 | 5.23 |
| Lab no.15 | 0.1 | 0.09 | 0.74 | 0.56 | 2.62 | 1.9 | 6.26 c | 2.24 c | 8.94 | 9.07 |

Pork in raw beef

| Nominal level (%) | 0.1 | 0.1 | 0.5 | 0.5 | 1 | 1 | 3 | 3 | 10 | 10 |
|-------------------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| Sample ID | PCR_23 | PCR_28 | PCR_30 | PCR_04 | PCR_27 | PCR_01 | PCR_16 | PCR_10 | PCR_29 | PCR_15 |
| Lab no.1 | 0.13 | 0.12 | 0.57 | 0.48 | 1.59 | 1.26 | 3.17 | 3.08 | 10.72 | 9.02 |
| Lab no.2 | 0.18 | 0.14 | 0.74 | 0.87 | 1.59 | 1.75 | 3.53 | 3.94 | 10.94 | 10.28 |
| Lab no.3 | 0.07 | 0.05 | 0.63 | 0.43 | 1.63 | 0.99 | 3.83 | 2.69 | 10.58 | 11.67 |
| Lab no.4 | 0.07 | 0.16 | 0.64 | 0.82 | 1.22 | 1.72 | 4.27 | 4.67 | 10.94 | 11.68 |
| Lab no.5 | 0.13 | 0.1 | 0.64 | 0.42 | 1.29 | 1.09 | 2.78 | 3.06 | 10.74 | 9.62 |
| Lab no.6 | 0.12 | 0.08 | 0.57 | 0.55 | 1.18 | 1.03 | 2.91 | 2.78 | 9.86 | 9.36 |
| Lab no.7 | 0.07 | 0.11 | 0.59 | 0.62 | 0.99 | 1.48 | 3.88 c | 1.89 c | 8.52 | 11.52 |
| Lab no.8 | 0.14 | 0.13 | 0.74 | 0.55 | 1.37 | 1.42 | 3.49 | 3.5 | 10.49 | 10.74 |
| Lab no.9 | 0.18 | 0.13 | 0.61 | 0.57 | 1.32 | 1.15 | 3.34 | 3.04 | 10.48 | 10.49 |
| Lab no.10 | 0.1 | 0.1 | 1 | 0.5 | 1 | 1 | 3 | 3 | 10 | 10 |
| Lab no.11 | 0.1 | 0.12 | 0.65 | 0.57 | 1.19 | 1.18 | 3.31 | 3.37 | 10.09 | 9.69 |
| Lab no.12 | 0.66 g | 0.48 g | 1.77 g | 1.29 g | 3.08 g | 2.77 | 6.15 g | 4.83 g | 16.3 g | 16.42 g |
| Lab no.13 | 0.08 | 0.09 | 0.49 | 0.5 | 1.1 | 1.11 | 2.56 | 2.63 | 9.6 | 7.76 |
| Lab no.14 | 0.03 | 0.05 | 0.33 | 0.51 | 0.58 | 1.01 | 2.57 | 2.57 | 6.12 g | 7.39 g |
| Lab no.15 | 0.09 | 0.08 | 0.88 | 0.85 | 1.83 | 1.26 | 3.35 c | 7.83 c | 40.18 c | 11.3 c |

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 |
| 6 | 0.998 | 0.998 | 0.995 | 0.998 | 0.998 | 0.992 |

| Lab number | Pork 1 | Pork 2 | Pork 3 | Horse 1 | Horse 2 | Horse 3 |
|------------|--------|--------|--------|---------|---------|---------|
| 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 |
| 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.