Inter-laboratory collaborative trial of real-time PCR method phase 2: Executive summary

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1. This project (FS430818) was initiated by the Food Standards Agency, UK, with support from the Department for Environment, Food and Rural Affairs, UK. The overall project describes the full international interlaboratory collaborative trial to define the performance characteristics of the real-time PCR method for horse and pork DNA in raw and processed beef matrix covering the range of concentrations 0.1-10% (w/w of raw meat).
2. The UK/EU Horse-meat issue of 2013, where a significant amount of horse DNA was found in a large number of beef meat products on sale at a supermarket store, prompted the development (Defra project FA0135) and interlaboratory validation (FSA project FS126001) of a real-time PCR approach for the quantitation of horse DNA in raw beef.
3. The real-time PCR approach was extended to develop three new methods for the quantitation of horse and pork DNA in raw and processed beef background (Defra project FA0171). A limited UK based ring-trial provided evidence of the fitness for purpose of the three new methods, applicable for DNA extracted from meat derived from horse and pork samples and demonstrated acceptable precision around the 1% (w/w) level for enforcement action. The methods can reliably distinguish between adventitious contamination at 0.1%, enforcement level at 1% and economically motivated adulteration at 10%.
4. This report is supplementary to the interim report against Objective 1.2 of the project which describes the validation of the method and associated verification of the test samples for the collaborative trial. Essential information is duplicated across both reports and this report, against Objective 5.4, remains the overall project report.
5. The interim report against Objective 1.2 of the project was initially reported in March 2022, to internally validate the previously-developed real-time PCR method for the quantification of horse and pork in beef (raw and processed). The interim report additionally described the validation of the CTAB method for extraction of the DNA, quantification of extracts against standard curves, and the application of the method to verify the homogeneity of the test samples.
6. Test samples comprised DNA extracts from three types of analyte/matrix combinations: horse in processed beef, pork in processed beef, pork in raw beef. Each sample type was prepared at five nominal concentration levels: 0.1%, 0.5%, 1%, 3%, 10% (w/w of raw meats initially combined). The collaborative trial design was that of blind duplicates, comprising a total of 30 test samples.
7. Laboratories were recruited in the UK and internationally to take part in the collaborative trial. The 15 laboratories that were recruited were sent test samples, necessary

consumables for undertaking the analysis and full instructions including SOP for the method in May 2022. All 15 laboratories had returned results by the time of the final July 2022 deadline, using a secure website facility.

8. Results were analysed according to established collaborative trial principles which initially involved the removal of non-compliant pairs (non-detects), Cochran's outlier removal and Grubbs' outlier removal. No more than three pairs of data needed to be removed per sample type.
9. The output of the data analysis was the measure of repeatability precision, reproducibility precision and critical differences. The pre-defined target precision was 25% relative standard deviation.
10. The ratio of observed precision to target precision was in the range 0.350 to 1.45 for repeatability and 0.371 to 1.43 for reproducibility. The collaborative trial has achieved acceptable precision for the method real-time PCR of DNA extracts for horse in processed beef, pork in processed beef and pork in raw beef.