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

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Gweld Inter-laboratory collaborative trial of real-time PCR method for the relative quantitation of horse DNA and pork DNA in raw and as PDF(Open in a new window) (1.17 MB)

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 limits 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 describes the interim objective 1.2 of project FS430818, to internally validate the previously-developed real-time PCR method for the quantification of horse and pork in beef (raw and processed).
5. Sample preparation comprised preparation of raw horse, pork and beef in clean laboratory facilities to avoid cross-contamination. Processing of the meat and meat combinations in the laboratory was done so that there was minimal DNA degradation by dehydration of the meat.
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).
7. The CTAB extraction method obtained DNA of sufficient quality and quantity for analysis. Eight-fold standard curves with a starting concentration of at least 40 ng/µl on raw 100% horse and pork covered a dynamic range to quantify all % w/w combinations of raw and processed horse in beef and pork in beef mixtures using the horse and pork standard curves respectively.

8. Reliable detection of 0.1% w/w contamination by horse or pork meat in a beef background in laboratory processed samples was successfully achieved.
9. The internal method validation was used to verify all the samples prepared for the subsequent full collaborative trial and confirmed their fitness for purpose in terms of their homogeneity and expected concentration.
10. The full collaborative trial was to be undertaken following completion of the internal validation and sample verification and is the subject of a separate report.