

# Inter-laboratory collaborative trial of real-time PCR method phase 1: conclusions and references

## Conclusions

We have internally verified the published method [5, 6] for horse and pork quantification using real-time PCR in the Fera laboratory. We have demonstrated that the CTAB method chosen is suitable to obtain DNA of sufficient quality and quantity. Eight-fold standard curves with a starting concentration of at least 40 ng/μl on raw 100% horse and pork cover a dynamic range of five points, and we were able 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.

Processing of the meat and meat combinations in the laboratory was done so that there was minimal DNA degradation by dehydration of the meat. This may differ to real world samples where meat has been cooked outside of the laboratory by a variety of methods that could accelerate DNA degradation. However, for the purpose of the collaborative trial, we have shown reliable detection of 0.1% w/w contamination by horse or pork meat in a beef background in laboratory processed samples.

The internal method validation in turn has allowed the verification of all the samples prepared for the full collaborative trial and confirmed their fitness for purpose in terms of their homogeneity and expected concentration. The outcome of the collaborative trial, as far as can be prepared for, is now dependent on the analysis by the participant laboratories in the trial.

## References

1. [Fera science Ltd, Standards & Accreditations](#), accessed 21/03/2022.
2. Lloyd's Register, [Learn about ISO 9001 Quality Management Systems \(QMS\)](#), accessed 21/03/2022.
3. Defra Report FA0135 (2013). Real-time PCR approach for quantitation of horse DNA and study into relevance of expression units (DNA/DNA and w/w tissue)
4. FSA Report FS126001 (2016). International collaborative trial of a real-time PCR method for the relative quantitation of horse DNA
5. Defra report FA0171 (2018). Validation of Methods to Quantify Horse and Pork Meat Adulteration in Raw and Processed Beef
6. Malcolm Burns, Gavin Nixon, Simon Cowen, Timothy Wilkes. International collaborative trial of a real-time PCR approach for the relative quantitation of horse DNA. Food and Nutrition Open Access, 2018, DOI: 10.31021/fnoa.20181113
7. CEN/TS 17329-1. Foodstuffs – General guidelines for the validation of qualitative real-time PCR methods – Part 1: Single-laboratory validation. (Final draft at January 2021.)
8. ISO/DIS 20813:2018. Molecular biomarker analysis – Methods of analysis for the detection and identification of animal species in foods and food products (nucleic acid-based methods) – General requirements and definitions