Inter-laboratory collaborative trial of real-time PCR method phase 2: conclusions and references

Conclusions

The analytical method for horse and pork DNA quantification using real-time PCR was internally validated and the CTAB method was found to be suitable to obtain DNA of sufficient quality and quantity. Using eight-fold standard curves covering the required dynamic range, all % w/w combinations of horse in processed beef and pork in raw and processed beef mixtures were quantified 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. For the purpose of the collaborative trial, there was reliable detection of 0.1% w/w contamination by horse or pork meat in a beef background in laboratory processed samples.

Using the validated method, all the samples prepared for the full collaborative trial confirmed their fitness for purpose in terms of their homogeneity and expected concentration. The samples were then dispatched to participants in the collaborative trial, with results returned by all participants.

Repeatability and reproducibility precision was determined for all sample types at all preparation levels, including the lowest 0.1% w/w contamination level, and for all the processed samples. The acceptability of the precision was determined via the target ratio of observed precision to target precision of 25% relative. The target ratios never exceeded 1.45, i.e. there was no unacceptably large variance. The target ratios reduced at higher preparation levels (towards adulteration levels) so the method would always be suitable to detect economic adulteration of meats, either raw or processed.

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