International collaborative trial of a Real-Time PCR method for the Relative Quantitation of Horse DNA

Area of research interest: Capabilities
Study duration: 2015-10-01
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Conducted by: LGC

Background

Following the UK/EU Horse-meat issue of 2013, where a significant amount of horse DNA was found in a beef burger product on sale at a supermarket store, a real-time PCR approach for quantitation of horse DNA was developed at LGC using Defra funding. The method was applicable for meat derived from different horse samples, demonstrated acceptable precision around the 1% (w/w) level for enforcement action, and was applicable for selected processed food materials.

This project is an international collaborative trial of the method based on International and European guidelines, (IUPAC (International Union of Pure and Applied Chemistry) and ENGL (European Network of GMO Laboratories) respectively) and was organised in order to evaluate the repeatability and reproducibility of the method within and between laboratories to provide evidence of its fitness for purpose.

Research approach

A total of seventeen laboratories participated in the trial, including nine official control laboratories and private labs in the UK, six laboratories from EU member states, and laboratories in Switzerland and the United States of America. Each of the participating laboratories were requested to return results based on the evaluation of five blindly labelled test samples (representing 0.1, 0.5, 1, 5, and 20 % (w/w) horse meat in a beef meat background) relative to a calibration curve. Test samples were provided as DNA extracted from gravimetrically prepared raw horse-meat in a raw beef background. Each sample was represented by four units in the experimental design, and each unit represented by triplicate PCR technical replicates.

The collated data from the collaborative trial was subject to statistical analysis, and significant outliers removed from subsequent analysis. Based on the remaining data, the mean values for the PCR efficiency and r-squared associated with the calibration curves for the horse assay were 94.1% and 0.998 respectively, and for the mammalian assay, 96.4% and 0.997 respectively. The values obtained provided evidence that the PCR efficiency and linearity for the calibration curves across all laboratories was good.

Results

The relative reproducibility standard deviation (RSD_R) was calculated as less than 18% across all test samples for the levels 0.5% to 20% (w/w) inclusive, and as 26% at the 0.1% (w/w) level. These values fulfil the acceptance criteria for the precision associated with a method subject to a collaborative trial as outlined in published ENGL (European Network of GMO Laboratories) guidance notes for minimum performance requirements for analytical methods.

Both the repeatability and reproducibility estimates from the collaborative trial provide evidence for the good precision of the method within and between laboratories. Given the good precision and trueness estimates associated with this method as evidenced by an international collaborative trial, it is a recommendation of this project that the method be considered for standardisation at an international level.

England, Northern Ireland and Wales

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