

# Inter-laboratory collaborative trial of real-time PCR method phase 1: Introduction

Following 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, a real-time PCR approach for the quantitation of horse DNA in raw beef was developed at LGC through Defra funding (project FA0135, **Real-time PCR approach for quantitation of horse DNA and study into relevance of expression units** (DNA/DNA and w/w tissue)) [3]. This method was validated through an international collaborative trial (project FS126001 - **International collaborative trial of a real-time PCR method for the relative quantitation of horse DNA**) by LGC and funded by the FSA [4]. The success of this validation study has enabled the method to be formally subjected to European Standardisation at CEN where it is currently undergoing standardisation through CEN Technical Committee CEN TC460 on Food Authenticity.

A requirement to expand the real-time PCR approach to include pork meat in both raw and processed beef has resulted in the development of three SOP methods for the quantitation of horse and pork DNA in raw and processed beef background. The study was funded by Defra under its Food Authenticity Programme (project FA0171, Validation of Methods to Quantify Horse and Pork Meat Adulteration in Raw and Processed Beef) [5, 6]. The project looked at three new real-time-PCR methods for the relative quantitation of horse DNA in processed beef products, and the relative quantitation of pork DNA in raw and processed beef products. It included the development, the in-house validation and a limited UK based ring-trial (involving four UK laboratories). The study provided evidence of the fitness for purpose of the three new methods, as qualified by the limited (four laboratory) ring-trial. All three methods are applicable for DNA extracted from meat derived from horse and pork samples and demonstrate acceptable precision around the 1% (w/w) level for enforcement action. Furthermore, they can reliably distinguish between adventitious contamination at 0.1%, enforcement level at 1% and economically motivated adulteration at 10%.

Tenders were invited to plan a full-scale inter-laboratory collaborative trial on the methods that were developed under Defra project FA0171 [5] and additionally published [6] on the quantitation of horse DNA in processed beef and pork DNA in raw and processed beef. This report describes the initial method validation prior to its use in verifying the samples prepared for the full collaborative trial. The preparation of the samples and their verification of homogeneity are described. This interim report was presented in draft form to the FSA and Defra in March 2022, at the mid-point of sample preparation for the trial. Successful method validation and demonstration of the sample preparation up to that point was confirmation for the project to proceed to the full trial stage.