Quantitation of DNA from meat species using digital PCR

Application of the QuantStudio 3D Digital PCR System for detection and relative quantitation of adulterant meat species

In this document we demonstrate:

- Digital PCR may provide more accurate sample concentration estimates based on absolute copy number detection, compared to qPCR.
- The Thermo Scientific™ RapidFinder™ Equine ID Kit and RapidFinder™ Quant Multi-Meat Set can be successfully used on the Applied Biosystems™ QuantStudio™ 3D Digital PCR System for the detection and relative quantitation of adulterant horse meat in beef, based on raw meat samples.

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The Government Chemist role was created in 1842 in the UK, to help safeguard the quality of public science and ensure accurate analytical measurements. This article describes some of the work which was conducted as part of the Government Chemist Programme 2014–2017 for investigating new DNA technologies.

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Introduction

In 2013, the Food Safety Authority of Ireland (FSAI) published findings regarding a significant proportion of horse DNA being found in beef burger products on sale at a UK supermarket. These findings prompted the UK Government to conduct UK-wide surveys of beef products—leading to a highly publicized and lengthy issue on meat adulteration that went on to involve much of Europe. An EU-wide survey of beef products found that 4.7% of the samples tested positive for horse meat or horse DNA [1]; highlighting the need for the development and maintenance of accurate methods for the detection and quantitation of adulterant meat species.

Background

Real-time PCR (qPCR) provides a sensitive and specific method for detection of meat adulteration that can also be quantitative. This approach relies on the use of a standard curve, but if calibrants are not of similar quality to the test samples (for example due to the composition and processing being different), accurate quantitation can be problematic. An alternative method, that remains both sensitive and specific but does not rely on a standard curve, is digital PCR (dPCR). Our digital PCR systems, such as the QuantStudio 3D Digital PCR System, facilitate absolute single-molecule detection. This is achieved by a limiting dilutions approach. According to this approach, the reaction is divided into 20,000 individual partitions, and the absolute copy number is determined based on statistical interpretation of the number of partitions where the target has been detected, compared to those where it has not been detected [2]. The present study set out to evaluate the application of the QuantStudio 3D Digital PCR System for the detection and quantitation of adulterant meat species in comparison to a real-time PCR system, the Applied Biosystems™ 7900HT Fast Real-Time PCR System.

Materials and methods

DNA was extracted from a gravimetrically prepared 1% weight for weight (w/w) lean, raw horse meat in lean, raw beef meat sample. The 1% (w/w) level was chosen as it was reflective of the level used for enforcement action in the EU during the horse meat crisis. Multiple technical replicates from this DNA extraction were analyzed using the RapidFinder Equine ID Kit, for the detection of equine mitochondrial DNA, in conjunction with the RapidFinder Quant Multi-Meat Set, for the detection of highly conserved mammalian mitochondrial DNA. Use of the two kits together enabled the percentage of equine DNA relative to the total mammalian DNA in a sample to be calculated. The RapidFinder kits were applied to both PCR instrument platforms (QuantStudio 3D Digital PCR and 7900HT Fast Real-Time PCR systems) with the sample analyzed in duplicate across four repeat qPCR plates and with a total of 18 QuantStudio™ 3D chips (9 chips for the Equine ID Kit and 9 chips for the RapidFinder Quant Multi-Meat Set, along with appropriate negative and positive controls).

Results

The mean quantity of horse DNA relative to beef DNA in the 1% horse in beef (w/w) sample was estimated by qPCR to be 0.73 ± 0.48% (measurement uncertainty based on a 95% confidence interval) and by the QuantStudio 3D system to be 0.99 ± 0.44% (Figure 1). Both estimates show good trueness to the expected value of 1% horse in beef, particularly so, as mitochondrial DNA targets were used. Both approaches demonstrate a high level of agreement, although there is less bias associated with the dPCR system. Both approaches also show similar measurement uncertainty levels which suggest that they are comparable in terms of precision.

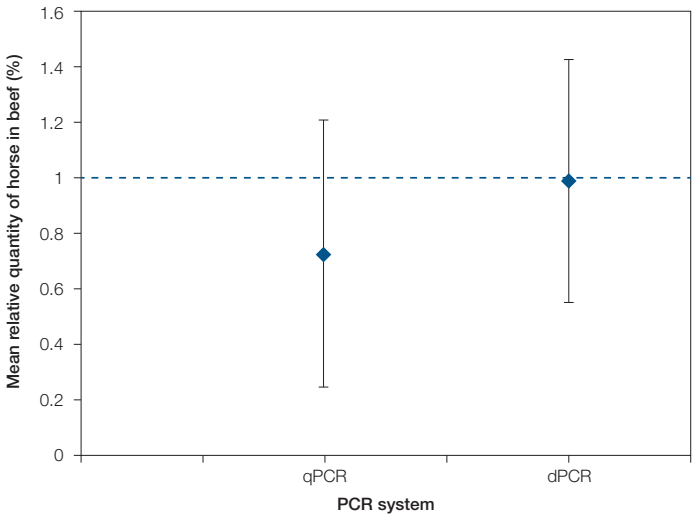

Figure 1. The mean quantity of horse DNA relative to beef DNA estimated using a real-time PCR system and the QuantStudio 3D Digital PCR System. The quantity of horse DNA relative to beef DNA in a gravimetrically prepared 1% horse in beef (w/w) sample was estimated using 4 real-time PCR plates and 18 QuantStudio 3D chips. Error bars represent the expanded measurement uncertainty based on a 95% CI.

Although there is a good level of agreement between the quantities of horse DNA relative to beef DNA determined by the two PCR systems, the two instruments appear to provide different absolute copy number estimates of beef DNA and horse DNA (Table 1). The qPCR-estimated copies of horse DNA is 1.7 times greater than the dPCR-estimate, and the copies of beef DNA estimated by qPCR is 2.3 times greater than the dPCR-estimate. This difference may be because of the different mechanisms used to estimate DNA copy numbers between qPCR and dPCR. For qPCR, copy numbers of the test samples are estimated relative to a calibration curve. Copy number counts are based on UV spec readings, estimated genome size, molar mass of nucleotides, and DNA mass concentration. In contrast, for dPCR, copy number estimates are based on absolute detection of single molecules; mitigating issues associated with the use of calibrants, such as matrix-matching and effective determination of nucleic acid concentration, which are necessary for qPCR-based approaches and can introduce measurement uncertainty. These factors potentially give dPCR greater sensitivity and precision than qPCR [2]—suggesting that the dPCR estimates of absolute copy number produced in this study may be more reliable than the qPCR estimates.

Conclusions

At the time of the 2013 horse meat issue, a level of 1% horse in beef (w/w) was suggested for enforcement action in the UK and EU [1]. The use of appropriate reference materials and quality controls were instrumental in affording confidence in these measurements. This study demonstrates that the QuantStudio 3D system was successfully used to determine the relative amount of horse meat in beef at this level—producing a relative quantity of horse in beef estimate with good concordance to the expected value, and with good agreement in terms of both trueness and precision to qPCR estimates. This study also demonstrated that the RapidFinder Equine ID Kit and Quant Multi-Meat Set can be successfully used with the QuantStudio 3D Digital PCR System for the detection and relative quantitation of adulterant horse meat in beef, with little or no optimization required.

Acknowledgements

The work described in this article was funded by the UK Department for Business, Innovation & Skills as part of the Government Chemist Programme 2014–2017.

Table 1. The estimated mean copy numbers of horse and beef DNA targets per microliter in the original stock solution of the 1% horse in beef (w/w) sample, based on a real-time PCR system and the QuantStudio 3D Digital PCR System. Copies of horse and beef DNA determined using qPCR were calculated as copies per PCR reaction and later converted to copies per microliter for comparison to dPCR estimates. The quantity of horse DNA relative to beef DNA estimated using qPCR is calculated using DNA copies per PCR reaction.

Instrument used	qPCR system		dPCR system	
Meat type copies/µL	Horse copies/µL	Beef copies/µL	Horse copies/µL	Beef copies/µL
Mean	26,128	3,435,602	15,725	1,473,897

References

1. Walker MJ, Burns M, Burns DT (2013) Horse meat in beef products – species substitution. *Journal of the Association of Public Analysts* 41:67–106.
2. Majumdar N, Wessel T, Marks J (2015) Digital PCR modeling for maximal sensitivity, dynamic range, and measurement precision. *PLoS One* 10(3): e0118833.

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Ordering information

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