

# Quantification of Pork, Chicken, Beef and Sheep Contents in Meat Products Using Duplex Real-Time PCR

## Supplementary Materials

**Table S1.** Composition of PT samples.

Sample name	Sample Description	Date of Distribution
FAPAS-PT1	The test material is raw lamb (94%) (freeze-dried) and has been contaminated with goat (3%) and pork (3%).	7.2022
FAPAS-PT2	The test material is raw beef (90%) (freeze-dried) and has been contaminated with equine (5%) (horse) and lamb (5%).	2.2022
FAPAS-PT3	The test material is raw chicken (92%) (freeze-dried) and has been contaminated with pork (3%) and turkey (5%).	4.2021

FAPAS: The Food Analysis Performance Assessment Scheme

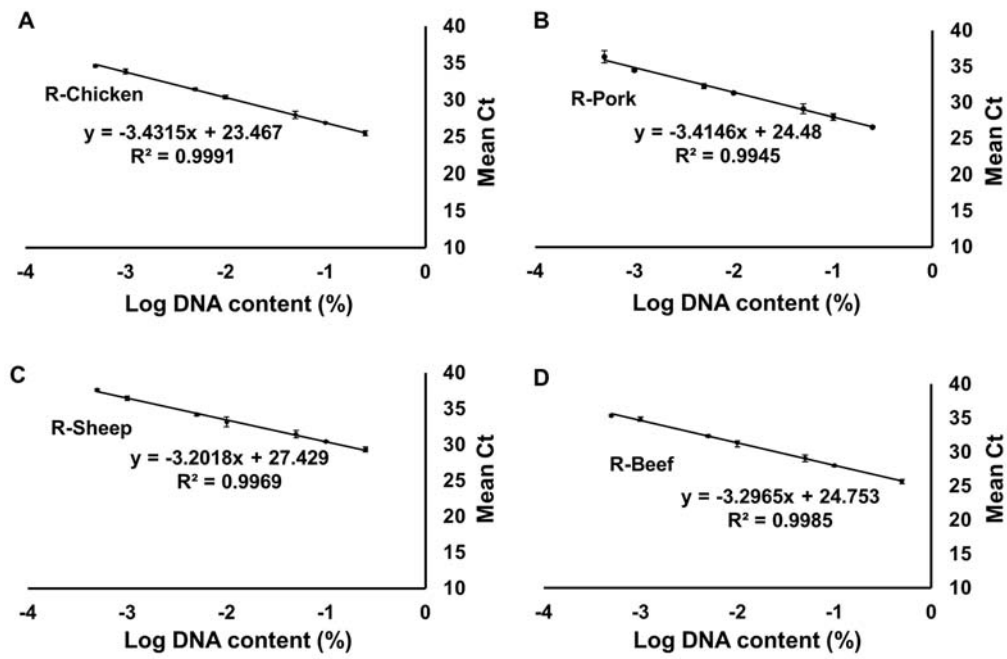
**Table S2.** Specificity of the real-time PCR system.

Species	Expected results					Tested results				
	Pork	Chicken	Beef	Sheep	PC	Pork	Chicken	Beef	Sheep	PC
Cattle ( <i>Bos taurus</i> )	ND	ND	D	ND	D	ND	ND	D	ND	D
Chicken ( <i>Gallus gallus</i> )	ND	D	ND	ND	D	ND	D	ND	ND	D
Crocodile ( <i>Crocodylus niloticus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Donkey ( <i>Equus asinus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Duck ( <i>Anatidae</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Goat ( <i>Capra hircus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Goose ( <i>Anserinae</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Hare ( <i>Lepus europaeus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Horse ( <i>Equus caballus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Kangaroo ( <i>Macropodidae</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Ostrich ( <i>Struthio camelus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Pig ( <i>Sus scrofa domestica</i> )	D	ND	ND	ND	D	D	ND	ND	ND	D
Rabbit ( <i>Oryctolagus cuniculus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Red deer ( <i>Cervus elaphus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Roe deer ( <i>Capreolus capreolus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Sheep ( <i>Ovis aries</i> )	ND	ND	ND	D	D	ND	ND	ND	D	D
Turkey ( <i>Meleagris gallopavo</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Wild boar ( <i>Sus scrofa scrofa</i> )	D	ND	ND	ND	D	D	ND	ND	ND	D
Black mustard ( <i>Brassica nigra</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Broccoli ( <i>Brassica oleracea</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Carrot ( <i>Daucus carota</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Celery ( <i>Apium graveolens</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
chili pepper ( <i>Capsicum sp.</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
garlic ( <i>Allium sativum</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
ginger ( <i>Zingiber officinale</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
Kailan ( <i>Brassica oleracea var. alboglabra</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
onion ( <i>Allium cepa</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
parsley ( <i>Petroselinum crispum</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
rapeseed ( <i>Brassica napus</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
rice ( <i>Oryza sativa</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
rosemary ( <i>Rosmarinus officinalis</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
rye ( <i>Secale cereale</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
tomato ( <i>Solanum lycopersicum</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
wheat ( <i>Triticum aestivum</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
cabbage ( <i>B rassica oleracea var. oleracea</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D
shiitake mushroom ( <i>Lentinula edodes</i> )	ND	ND	ND	ND	D	ND	ND	ND	ND	D

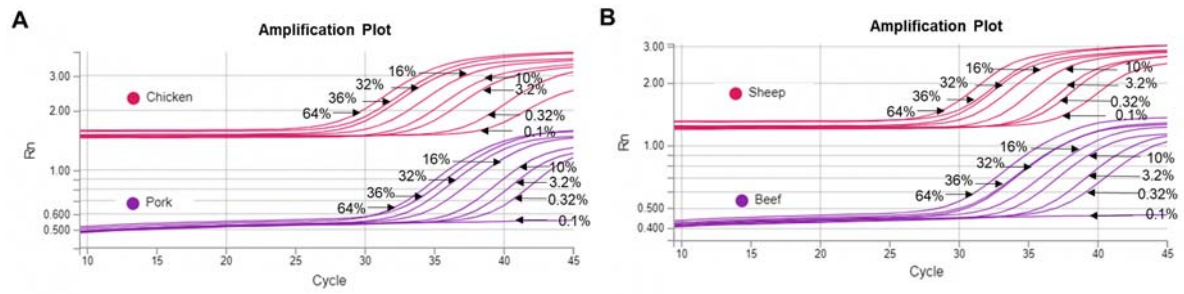
PC: internal positive amplification control on the 18S rRNA gene; ND: not detected. The genomic DNA for cattle (*Bos taurus*), chicken (*Gallus gallus*), pig (*Sus scrofa domestica*), sheep (*Ovis aries*) and duck (*Anatidae*) were extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany); genomic DNA from other common food ingredients: black mustard (*Brassica nigra*), broccoli (*Brassica oleracea*), carrot (*Daucus carota*), celery (*Apium graveolens*), chili pepper (*Capsicum sp.*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), Kailan (*Brassica oleracea var. alboglabra*), onion (*Allium cepa*), parsley (*Petroselinum crispum*), rapeseed (*Brassica napus*), rice (*Oryza sativa*), rosemary (*Rosmarinus officinalis*), rye (*Secale cereale*), tomato (*Solanum lycopersicum*), wheat (*Triticum aestivum*), cabbage (*B rassica oleracea var. oleracea*), and shiitake mushroom (*Lentinula edodes*) were extracted using CTAB method described in section 2.2; the genomic DNA solution for crocodile (*Crocodylus niloticus*), donkey (*Equus asinus*), goat (*Capra hircus*), goose (*Anserinae*), hare (*Lepus europaeus*), horse (*Equus caballus*), kangaroo (*Macropodidae*), ostrich (*Struthio camelus*), rabbit (*Oryctolagus cuniculus*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), turkey (*Meleagris gallopavo*) and wild boar (*Sus scrofa scrofa*) were purchased from Eurofins Scientific (France).

**Table S3.** Performance comparison of our duplex qPCR assays to other reported method.

Parameters	Duplex qPCR developed in this study	Multiplex qPCR reported by Köppel R.et al. 2013 [24]	Multiplex qPCR reported by Köppel R.et al. 2020 [25]
Amplification efficiencies %	98.7 - 104.5	59 - 107	89 - 97
Correlation R <sup>2</sup>	0.99 - 1.0	0.96 - 0.98	0.98 - 1.0
Precision ± %	2.45 – 24.58	15 - 24	4.09-103.23
Accuracy ± %	0.14 – 24.07	4.1 - 167.7	1.0 – 102.1



**Figure S1.** Standard curves plotted by the mean Ct values against 7 dilution series (25%, 10%, 5%, 1%, 0.5%, 0.1% and 0.05%) using singplex qPCR for chicken (A), pork (B), sheep (C) and beef (D). A DNA mixture consisting of 25% pork, 25% chicken, 25% beef and 25% sheep (20 ng/ $\mu$ L) was serially diluted in herring sperm DNA solution (20 ng/ $\mu$ L) and analyzed in 6 replicates.



**Figure S2.** The real-time PCR data of amplification curves for boiled meat using serial diluted DNA. (A) Amplification curves for boiled chicken and pork; (B) Amplification curves for boiled beef and sheep. For serially diluted DNA, 0.32 ng of DNA were detected for all boiled meat species.