BIPM Capacity Building & Knowledge Transfer Programme

2021 BIPM - TÜBİTAK UME Project Placement

REPORT

Project Name	Development of certified reference materials and methods for meat species identification
Description	This project developed the meat certified reference materials and analytical methods for meat species identification using qPCR and dPCR
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Date	1st Oct – 24th Dec 2021

Motivation & Introduction

The incidences of meat adulteration have been increasingly reported and become a globally widespread issue. The intentional meat substitution could be for reducing the cost by using the cheaper meat or misrepresentation of food ingredient labelling. These problems emphasised the need of accurate analytical methods and certified reference materials (CRMs) for the detection and quantification of meat adulteration. CRMs are important tools to validate analytical measurement procedures, to control the quality of measurement results and to establish the metrological traceability of measurement results. Currently, the CRM in meat adulteration sectors is inadequate and limit only for few kinds of meat. Hence, there is a necessity for developing and producing more varieties of CRMs to encourage the quality assurance and quality control in analytical laboratories for determination of meat adulteration.

Presently, Bioanalysis group, NIMT is collaborating with TÜBİTAK UME on organizing the inter-laboratory comparison of CCQM K86.d/P113.5 in pork quantification in beef background. In this programme, we would like to extend the scope of collaboration on developing new measurement methods and CRMs for other type of meats for meat identification including pork, beef, horse, goat, sheep and chicken. Participating in this project, therefore, would be a great opportunity to continue our collaboration. The tentative scope of work covered on development and validation of the methods and development of a candidate certified reference materials compliance with the ISO 17034.

Research

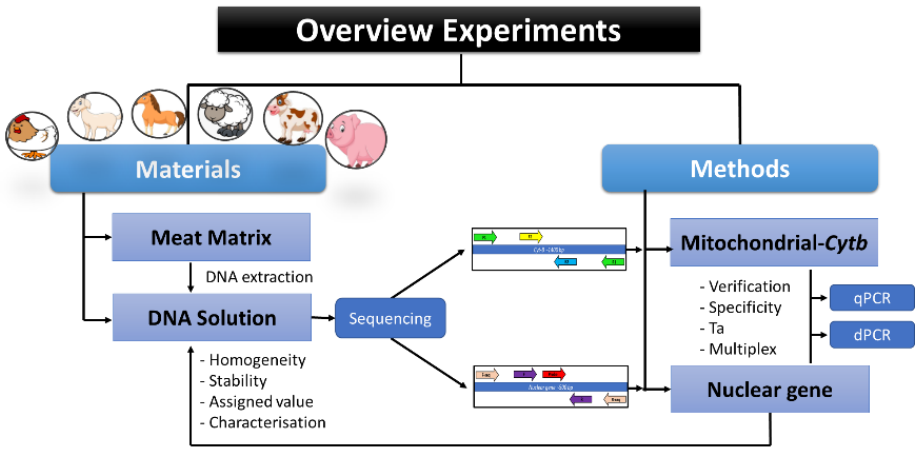

Fig 1: The overview of the meat identification experiments.

Part I: Development of candidate reference materials for meat adulteration in compliance with the ISO 17034

1. TÜBİTAK UME provided the training on how to prepare meat CRMs according to ISO 17034, particularly lyophilization technique and the material processing to control or minimise the sources of contamination.
2. The DNAs from six different meat matrixes were extracted by the salt method that was developed by UME. Ten bottles of each meat type were randomly selected and each was extracted in duplicate. Therefore, the total of sample DNA extraction were 120 replicates for 6 meat types.
3. To identify the meat species, the Sanger DNA sequencing method was used.
   - The primers were designed into two groups: nuclear genes and mitochondrial genes-*cytb* (Fig 2).
   - There were six of meat species including pork, beef, horse, goat, chicken, and sheep
   - The forward and reverse primers were designed to cover the nuclear target gene (ISO/TS 20224: 2020) with approximately 500 bp of amplicon size (Fig 2A), while for the *cytb* target were about 1400 bp (Fig 2B) and then the PCR were sent for sequencing using 4 different primers to sequence both strands.

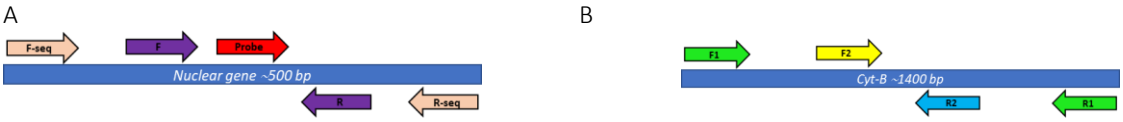

Fig 2: Designed primers for sequencing. Primers amplify the nuclear target gene (A) and *cytb* (B).

Part II: Development and validation of methods for meat species identification

To identify and certify the meat species, quantitative real time polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) were used. The assays were divided into two group; targeting nuclear genes (ISO/TS 20224: 2020) and mitochondrial gene according to the publications.

1. qPCR
   - To verify the method in qPCR, the selectivity (cross reactivity) of the assays was tested against the individual meat DNA and mixed DNA (mixed six DNA meat species). The results showed that in the nuclear gene assays, only sheep assay was non-specifically produced positive signal to goat DNA but at very late Ct's (Fig 3A) as the same non-specific signals were found in beef, goat, and sheep mitochondrial (*cytb)* assays, (Fig 3B).
   - To optimize DNA annealing temperature, the annealing temperature (Ta) was tested using a gradient qPCR (55-67 °C) and the results showed that the sheep assay was specific to their target at 63 °C (Table 1) for nuclear gene assays, while the mitochondrial (*cytb)* assays, since the positive signal was detected around Ct:20, the non-specific signal was detected around Ct:30, it might be possible both to end PCR at 30 cycles or dilute the DNA 1000 times to avoid non-specificity.

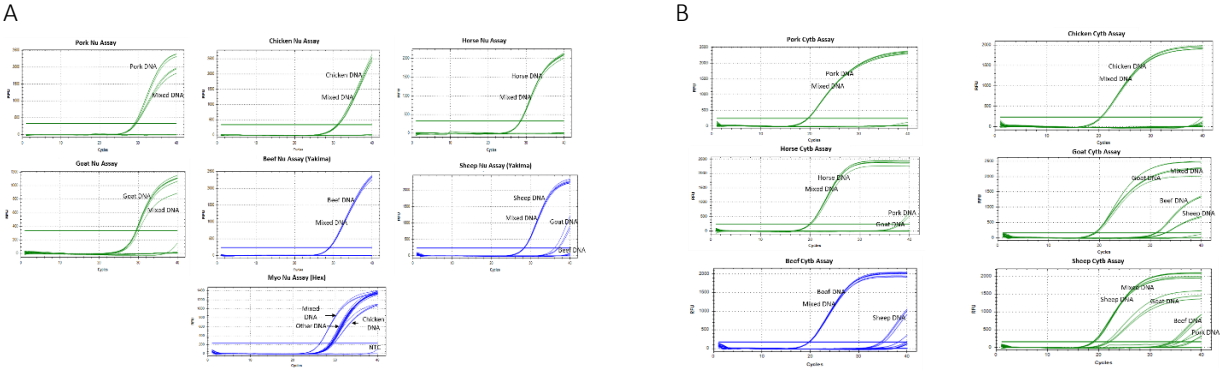

Fig 3: The amplification curves of testing the selectivity of assays against meat DNA. A) the nuclear assays and B) mitochondrial (*cytb)* assays were performed in qPCR.

2. ddPCR
   - Duplex assays (nuclear and myostatin assays) were tested against meat DNA. The results was shown in Fig 4. However, as the limitation of time, the duplex assays and *cytb* assays with ddPCR will be further investigated.

Table 1. The gradient qPCR (55-67 °C) to optimise the annealing temperature of nuclear gene assays.

Assay	Pork	Chicken	Horse	Goat	Beef-Yakima	Sheep-Yakima		
40 cycles/DNA	Pork	Chicken	Horse	Goat	Beef	Goat DNA	Sheep DNA	Mixed
Ta (°C)/Detected	Pork	Chicken	Horse	Goat	Beef	Goat DNA	Sheep DNA	Mixed
66.7	29.32	28.89	30.99	34.68	29.30	N/A	32.82	32.40
66.1	29.03	29.06	30.11	32.76	29.18	N/A	32.17	31.99
64.9	28.88	29.26	29.17	30.07	29.10	N/A	30.92	30.58
62.7	28.82	29.77	27.93	28.51	28.93	N/A	28.77	28.82
60.0	29.07	30.44	28.02	28.32	28.84	34.26	28.10	28.17
57.9	29.01	31.31	28.16	28.72	29.05	31.12	28.24	28.55
56.4	29.11	31.53	28.19	29.11	29.25	30.15	28.24	28.71
55.7	28.51	31.70	28.19	28.84	29.33	29.75	27.80	28.74

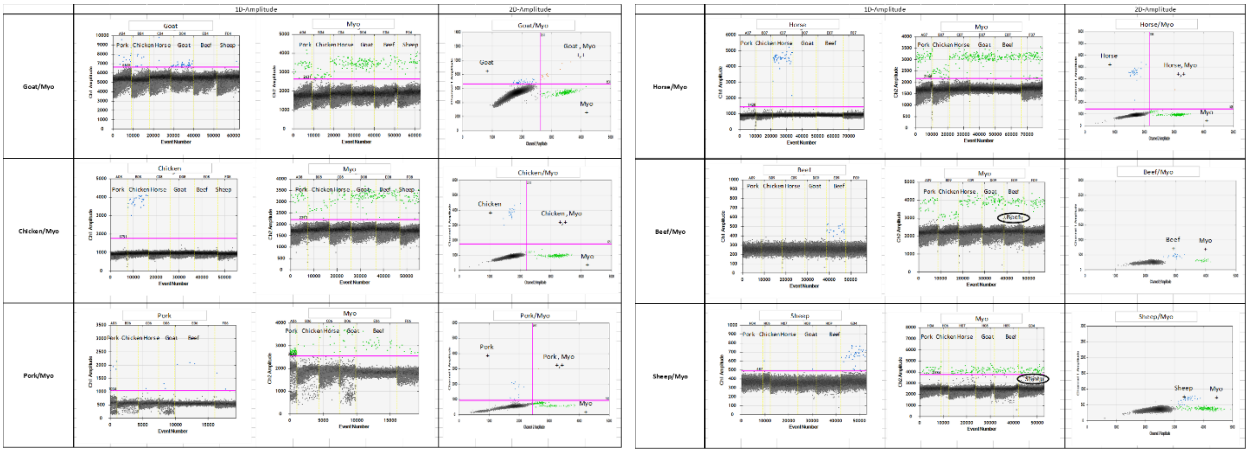

Fig 4: Duplex ddPCR results in 1D and 2D plot. The duplex assays including the nuclear gene target assay together with myostatin gene were performed in ddPCR.

Conclusions and Future Work

Participation in this programme is a precious experience that will allow me to expand my current abilities in food metrology fields. Without doubt, the knowledge, experiences and networks gained from this programme will enable not only me but also my institute, NIMT, to enhance the metrology researches and develop the standard in biology field especially in meat identification. I will transfer the exchanging knowledge and experience on method development and reference material production to NIMT's bioanalysis staffs particularly in the DNA extraction method (salt method) and meat identification method. Although, the works have not been finished yet as the limitation of time, TÜBİTAK UME and NIMT will continue our collaboration in developing the meat identification method by ddPCR.

Acknowledgements

I would like to thank BIPM and TÜBİTAK UME for providing the great opportunity in training programme, and NIMT for funding support. I would like to express gratitude my tremendous mentor, Dr. Muslum Akgoz, head of Bioanalysis Laboratory, who gave brilliant comments and suggestions for me. A special thanks to Dr. Burhanettin Yalcinkaya for always support and assistance me not only in the laboratory experiments but also in living life in Turkey. I am also very grateful to all who have contributed to the works and co-workers (Bioanalysis team, UME), thanks for all the helpful advice during this period and for creating a very good working environment.