Road map for the harmonisation of DNA meat speciation testing

Timothy Wilkes (LGC)
Government Chemist Conference 2018
Food chain resilience in a changing world, 13 - 14 June

Presentation overview

- Highlight some of the key issues associated with harmonisation of results for meat species detection and quantitation using DNA (PCR) based approaches
- Illustrate this by using detection of horse meat as an example (LGC, Defra and FSA project initiatives)
  - Road Map
- Traditional PCR based approaches (molecular biology):
  - Development of a real-time PCR approach for quantitation of horse DNA
  - Applicability to processed foods
  - International Collaborative trial of method
- . . . and beyond (additional approaches)
- Summary

Introduction

Horsemeat incident 2013

- 15th January 2013: Food Safety Authority of Ireland (FSAI) reported that a significant amount of horse DNA had been found in beef burger product on sale at a local supermarket

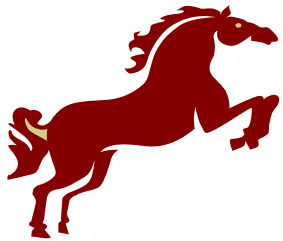
Background

- 16th January 2013 – Food Standards Agency (FSA) issues four-point plan for the investigation:
  1. Urgent review of the traceability of the food products identified in the FSAI survey
  2. Explore methodology used
  3. Consider whether any legal action is appropriate
  4. Work with Defra on a UK-wide survey
- In response to the 2013 EU horse-meat issue, Defra/FSA commissioned a UK survey of beef products
- EU/Global issue - Illegal substitution of beef with horse
- UK Government commissioned an independent review into the integrity and assurance of the food supply network in response to these findings

How did GC help?

GC assisted Government on all aspects of the four-point plan

- GC worked with FSA/Defra and provided advice on methods for determination of horse meat as part of the UK survey of beef products
- Advised on issues associated with threshold labelling (1%)
- Attended EU consultative meeting for 2nd round of horse-meat testing (Brussels) with FSA representing UK expert laboratory
- Members of Defra's AMWG and AMWG-TSG
- Project to establish whether species cross contamination occurs in UK meat processing plants during the GMP production of mince meat
- Analysed 7 referee cases related to meat speciation in 2013
  - Horse, beef, pork, lamb



LGC projects

In response to the 2013 horse meat issue, UK Government commissioned six projects at LGC:

- 2013
  - Defra Project FA0134: Method verification of the LOD associated with the UK Survey on Horse-meat
  - Defra project FA0135: Development of a real-time Polymerase Chain Reaction (PCR) method for the quantitation of horse DNA
- 2014
  - Defra project FA0146: Method validation of the real-time PCR method for quantitation of horse DNA
  - Defra project FA0157: Evaluation of quantitative molecular biology methods
- 2015
  - Defra project FA0162: GC Knowledge transfer event on the real-time Polymerase Reaction PCR for food authenticity testing inclusive of quantitation of horse DNA
  - FSA project FS126001: International Collaborative Trial of a Real-Time PCR Method for the Relative Quantitation of Horse DNA

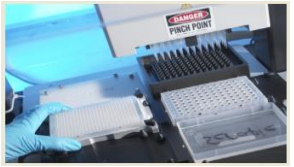
Defra Project (FA0135)

“Development of a real-time PCR approach for quantitation of horse DNA”

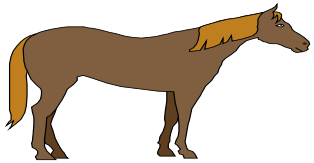
Introduction

Defra Project (FA0135) "Development of a real-time PCR approach for the quantitation of horse DNA"

- Aim of the work: Develop a real-time PCR approach for the quantitation of horse DNA
- Two nuclear DNA targets were chosen:
  - Horse specific target
  - General reference target for any mammalian DNA
- Meat samples (LGC Standards):
  - Authenticated for species identity using real-time PCR, ELISA and DNA sequencing
- Meat samples used to produce a range of w/w tissue gravimetric materials
  - Raw horse meat in a background of raw beef (meat) on a gravimetric w/w basis
- Subject to single-lab validation
- Tested on raw meat samples



Calibration



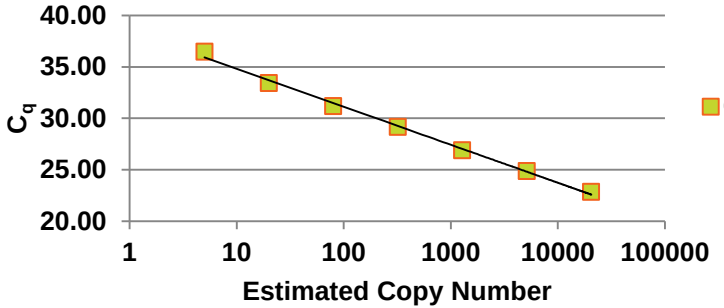
Sample

Extract DNA

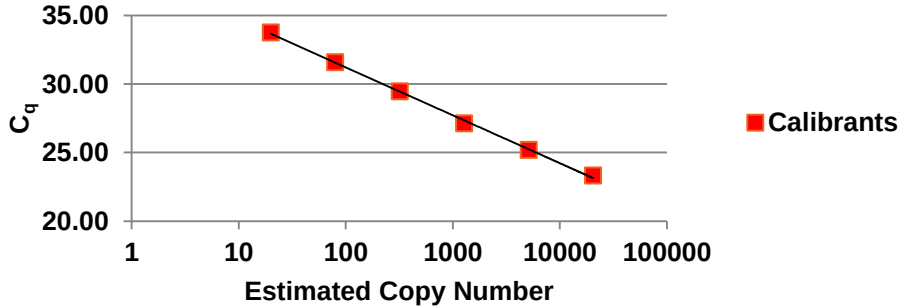
Dilute DNA

qPCR

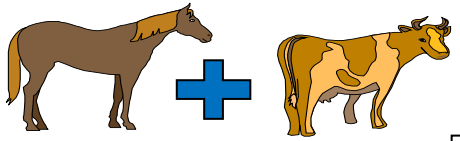
Horse Assay



Mammalian Reference Assay



Test sample evaluation

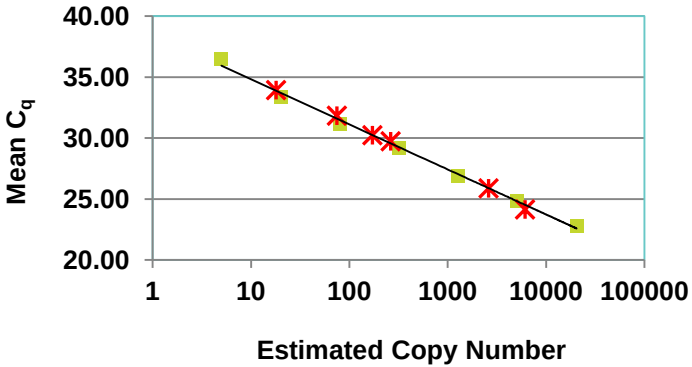


Test Samples
(w/w)

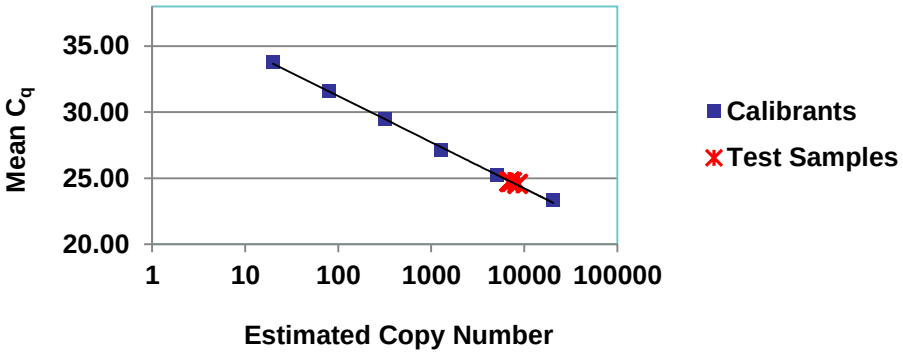
Extract DNA

qPCR

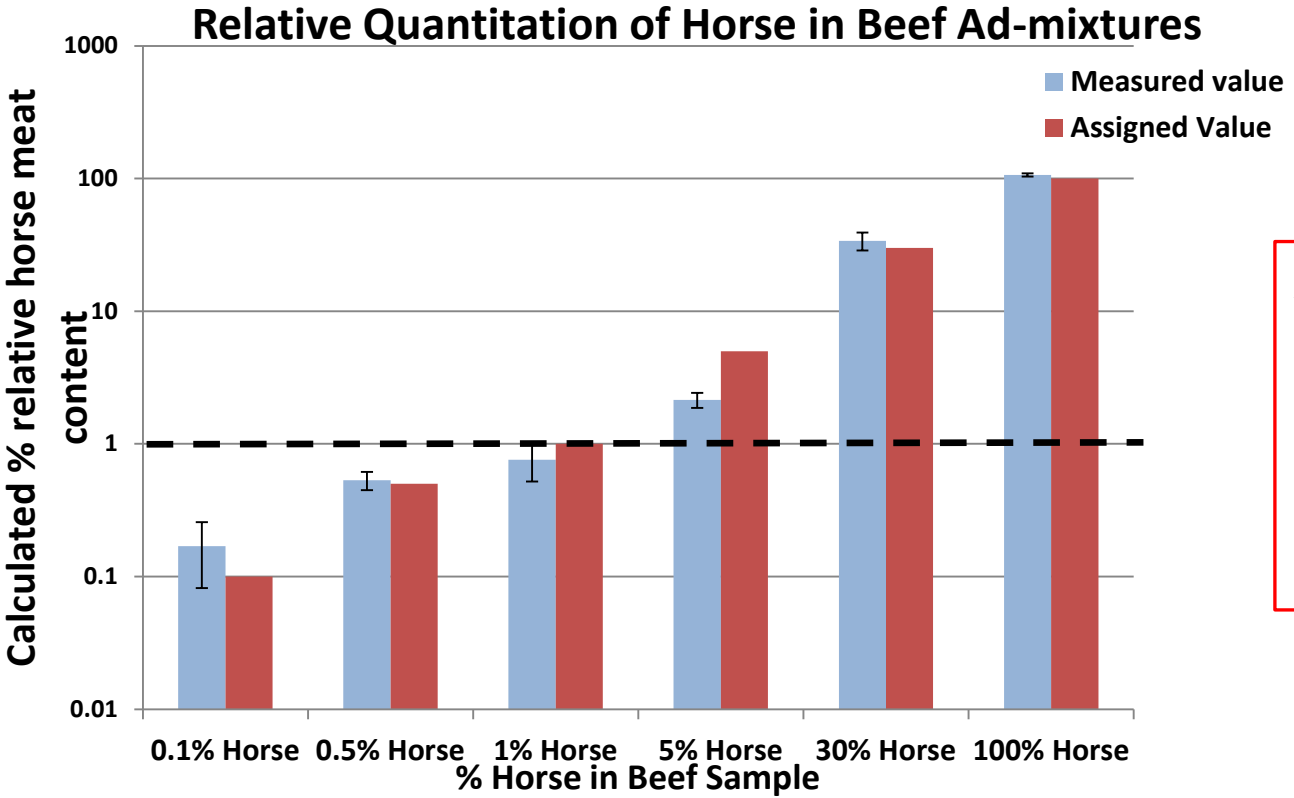
Horse Assay



Mammalian Reference Assay



Results



Analytical precision at the 1% (w/w) level for enforcement action:

1.03 ± 0.38 % (w/w)

- Real-time PCR approach had demonstrated good accuracy in estimating the levels of horse present in beef samples based on raw meat materials

Defra project (FA0157)

“Evaluation of Quantitative Molecular Biology Methods”

Introduction

- One of project objectives
  - Evaluate the applicability of selected PCR based methods for the reliable quantitation of ingredients in (processed) food stuffs
- LGC (Lead contractors)
  - Campden BRI; Royal Holloway
- PCR core technologies
  - Real-time PCR (ABI 7900HT)
  - Digital PCR (Bio-Rad QX200™)
- Assays
  - Horse in beef
- Test materials
  - 0.1 %, 1 %, 10 % (w/w) admixtures

ROYAL HOLLOWAY UNIVERSITY OF LONDON

Campden BRI
food and drink innovation

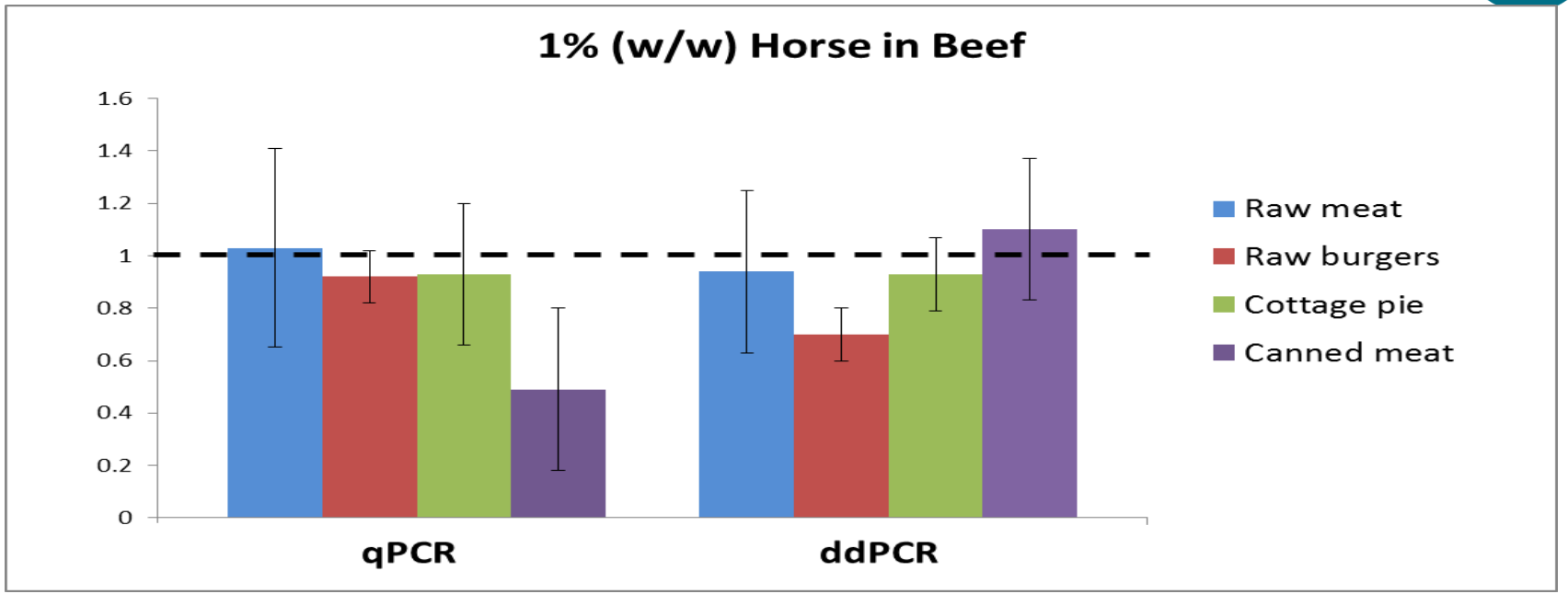
Department for Environment Food & Rural Affairs

Strategy

- Quantitative determination of adulterant content in processed food materials
- Campden BRI provided sample types (industry standard recipes):
  - Raw meat admixture (comparator)
  - Raw burger (raw meat)
  - Cottage pie (ready meal)
  - Canned beef (retorted)
- Samples made from scratch:
  - Appropriate amount of horse meat added to beef, and whole sample treated and processed according to industry standard recipes

Cottage pie sample type

DNA results for the 1% (w/w) samples



Comparison of horse (in beef)) qPCR and ddPCR assays using sample matrices at the 1% (w/w) level for enforcement action. Data based on two extractions, comprising six independent technical replicates per extraction. Values represent estimated mean percentage adulteration level with an expanded measurement uncertainty (based on a 95% confidence interval).

DNA results

- Analytical precision at the 1% (w/w) adulteration level for enforcement action (excluding the canned meat matrix):
  - qPCR (CV): 8 – 27%; ddPCR (CV): 10 – 24%
- Canned meat samples:
  - qPCR (CV): 45%; ddPCR (CV): 18%
- ddPCR generally showed tighter precision estimates:
  - Particularly at the 0.1% (w/w) levels
  - Highly processed food materials
- All test admixtures repeatedly detected and quantified:
  - Exception of the 0.1% (w/w) canned meat sample using the horse qPCR approach
- Demonstrates:
  - Utilisation of ddPCR for sensitive trace detection
  - Robust and sensitive nature of the quantitative PCR methodologies used
  - qPCR performed better than expected: careful selection of targets and assays

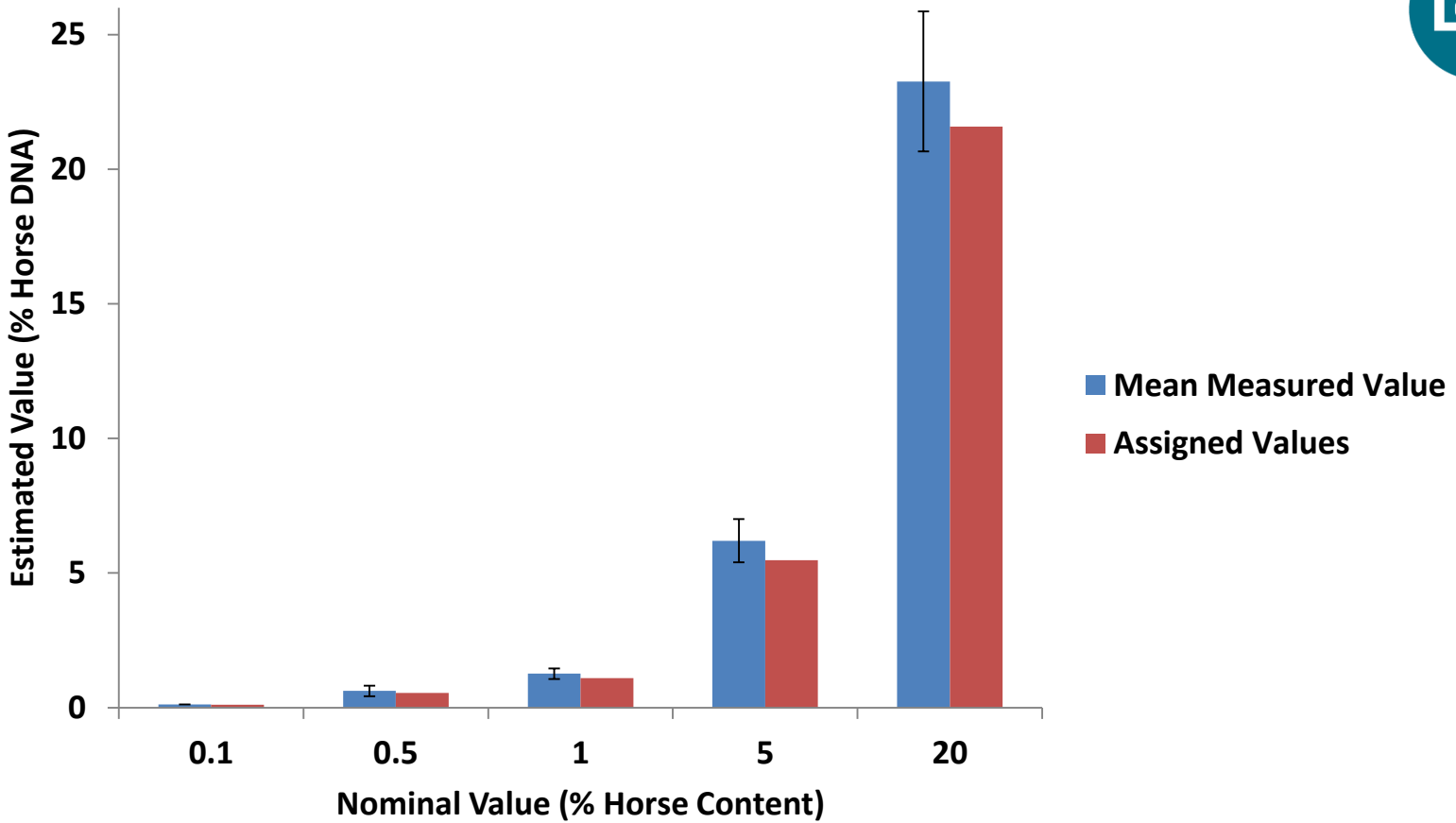
FSA Project (FS126001)

“An International Collaborative Trial of a Real-Time PCR Method for the Relative Quantitation of Horse DNA”

Basis of the Collaborative Trial

- Principle Aim:
  - To evaluate the repeatability and reproducibility of the method within and between laboratories
- Trial based on guidance provided by International Union of Pure and Applied Chemistry (IUPAC)
  - Protocol for the design, conduct and interpretation of collaborative studies
- Trial widely advertised internationally and open to all analytical laboratories
- Forty seven laboratories expressed interest in participating
  - Thirteen laboratories participated in the main trial
- Test samples
  - Five blindly labelled test samples
    - DNA extracted from 0.1, 0.5, 1.0, 5.0 and 20 % (w/w) horse meat in a beef meat background
    - Each sample represented by 12 replicates split across two experimental plates

Method Performance based on 13 labs



* Error bars encompass 2 x repeatability standard deviation (S_r)

Evaluation of critical method performance criteria

Measurement	Estimate from Collaborative Trial		Acceptance criteria (IUPAC/ENGL)	
	0.1% (w/w)	≥ 0.5% (w/w)	0.1% (w/w)	≥ 0.5% (w/w)
Relative repeatability standard deviation (RSD_r)	15%	9%	< 25%	< 25%
Relative reproducibility standard deviation (RSD_R)	26%	18%	<50%	<35%

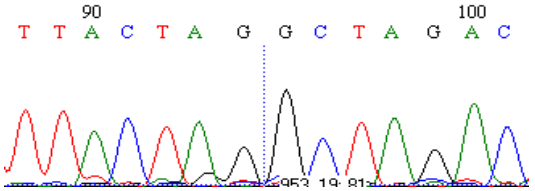
- Conclusions
  - Excellent repeatability and reproducibility estimates from the CT provide evidence for the good precision of the method within and between laboratories
  - Shows very little dispersion around the expected value
  - Values fulfil the acceptance criteria for the precision associated with a method subject to a Collaborative Trial
- Next steps:
  - Consider for standardisation at an international level through relevant CEN/ISO committee

Emerging technologies

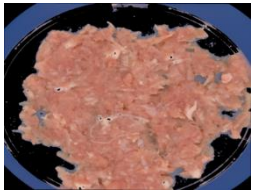
Additional approaches

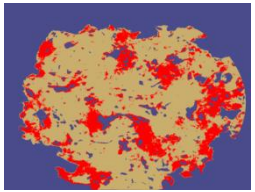
Other approaches being investigated for their potential for meat quantitation:

- DNA sequencing
  - NGS e.g. metabarcoding, relative abundance of different PCR amplicon populations
- dPCR
  - Absolute single molecule detection; calibration curve
  - Independent test of the size of different genomes (e.g. for a given weight of DNA what does this equate to in terms of equine or bovine genomes)
  - Enables conversion between percentage content based on mass/mass and copy number/copy number in some instances
- Multispectral imaging
  - Successfully applied for meat spoilage testing
  - Mixtures of meats, presence of offal



Bio-Rad QX200™ Droplet Digital™ PCR System (Bio-Rad Laboratories, Inc.)

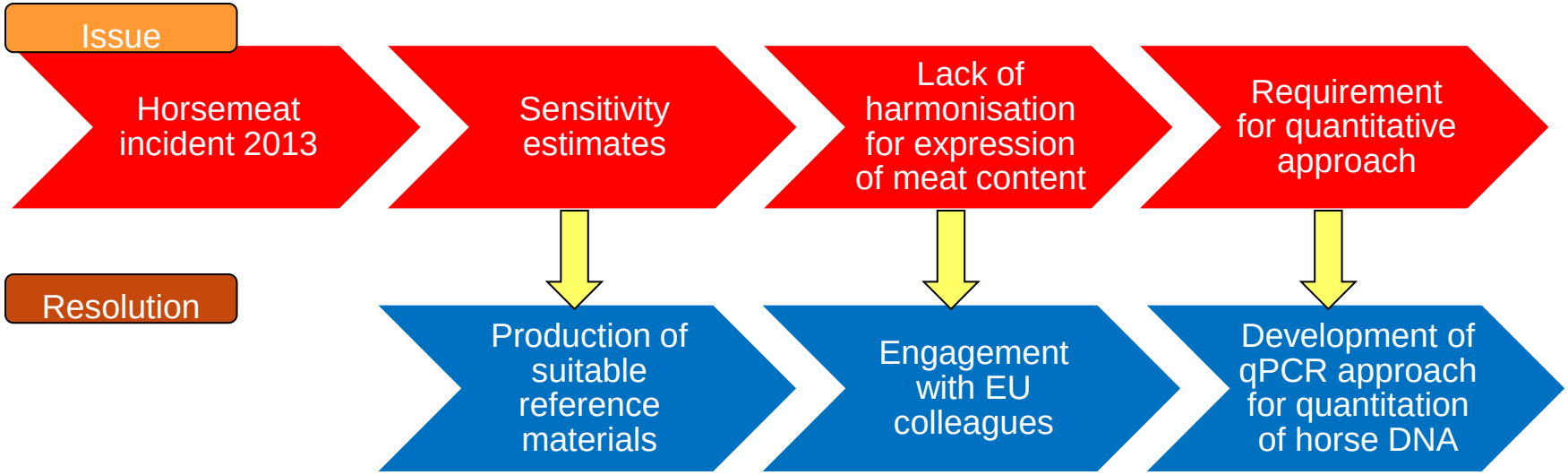




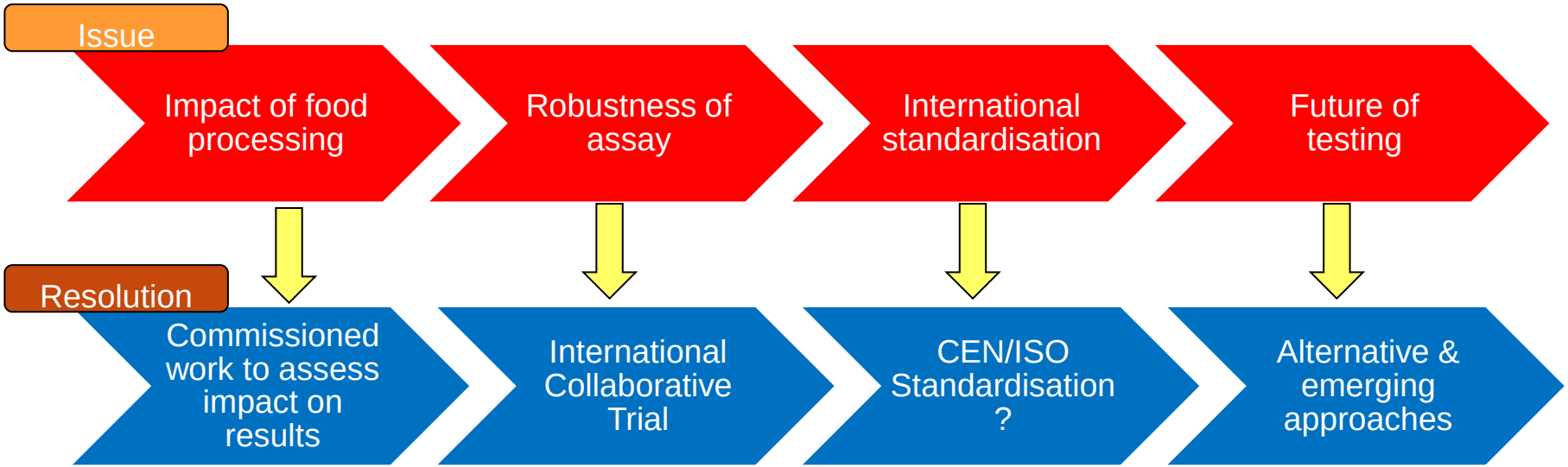
Summary and Road Map

Example Road Map

- Example navigational route to reach goal: towards harmonisation



Example Road Map (contd.)



Summary on meat quantitation

- The EU and UK horse meat incident highlighted a number of important issues
- Reinforced through the publication of:
  - Elliott Review in Sept 2014 “Integrity and assurance of food supply networks”
  - Defra’s AMWG response to Elliott review (March 2015)
- There is a lack of harmonised approaches for quantitating the level of meat adulteration
- Traceability of sources of materials/ingredients used in foods is a prerequisite
- A demonstrable need to invest in analytical techniques and strategies for the detection and quantitation of meat species:
  - Development (R&D)
  - Maintain these approaches and adapt as necessary

What goes around…..

theguardian

News | Sport | Comment | Culture | Business | Money | Life & style

News › UK news › Horsemeat scandal

Horsemeat scandal: EU ministers want faster action on meat labelling

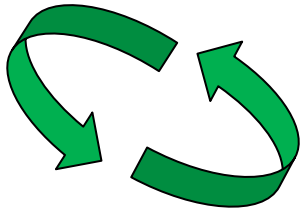
A European Commission report on tougher rules about origins of frozen beef products is expected but not until the end of 2013

James Meikle
The Guardian, Sunday 24 February 2013 19.22 GMT
Jump to comments (49)

Horsemeat found in frozen beef products has destroyed the trust many people placed in their local supermarkets. Photograph: Luke Macgregor/Reuters

Owen Paterson, the environment secretary, will be among ministers from across the EU pressing on Monday for speedier action on introducing country-of-origin labelling for processed beef and other meat products as they struggle to get a grip on the horsemeat scandal.

The Guardian
24th February 2013
James Meikle



The Guardian
1st April 1999
James Meikle

Label warning to meat industry

James Meikle

THE Government yesterday told the food industry to improve hygiene and labelling standards for sausages, burgers, pies, pat'es and prepared recipe dishes after tests revealed that nearly one in six contained undeclared types of meat.

Pork was detected in beef sausages and pies, turkey in chicken products and chicken in pork products, in a national survey of labelling claims by the Ministry of Agriculture.

Big retailers and manufacturers, including the main supermarkets, were among companies alleged to have failed to acknowledge all ingredients – although the

out anybody, big or small. We have been fair and double-checked the protocols, research and results."

The tests were designed to check claims, not food safety, but the large failure rate gave cause for concern. "We would not expect to see this repeated in another survey in a couple of years."

He contrasted the results with the efforts of orange juice manufacturers, which had made big improvements in the authenticity of their product and ended adulteration with sugar, malic acid or peel and pulp extracts.

Mr Rooker said: "It can't but help consumer confidence if consumers know that ingre-

Acknowledgements

- LGC
  - Malcolm Burns, Principal Scientist and special advisor to the Government Chemist
  - Gavin Nixon
  - Claire Bushell
  - Michael Walker
  - Kirstin Gray

http://www.foodauthenticity.uk/

FoodAuthenticity
A Virtual Network for Food Authenticity Analysis

Department for Environment Food & Rural Affairs

Department for Business, Energy & Industrial Strategy

FUNDED BY BEIS

Food Standards Agency
food.gov.uk

Thank you for listening

Contact details:

Timothy Wilkes

Timothy.Wilkes@lgcgroup.com

0208 943 7000